The Journal of
CLINICAL ENDOCRINOLOGY
AND METABOLISM

VOLUME 13 OCTOBER, 1953 NUMBER 10
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STUDIES IN NEPHROSIS: CHEMICAL CORTICOIDS, SALT-RETAINING FACTOR, AND EFFECT OF ACTH*

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INTRODUCTION

THE accumulation of edema fluid in patients suffering from the nephrotic syndrome, and the mechanism of its production, have been the subject of considerable speculation and research. According to Barnett (1) who has recently reviewed existing knowledge of the pathogenesis of this type of edema, there is evidence that a glomerular-tubular imbalance exists in the kidney in the nephrotic state. The preponderance of tubular function may depend on relatively decreased glomerular function, due either to pathologic lesions in the glomeruli or to "circulatory insufficiency" secondary to decreased serum osmotic pressure. Alternatively, it may indicate true tubular overfunction in response to excessive stimulation by posterior pituitary antidiuretic hormone, or adrenocortical salt-retaining hormone. The present study was undertaken with the object of further investigating adrenal function in this disease.

Received for publication February 25, 1953.

* The expenses incurred by these studies were defrayed by grants from the National Research Council of Canada to the Department of Pediatrics, McGill University, and to Dr. J. S. L. Browne.

The ACTH used was supplied by the National Research Council to the Department of Pediatrics, McGill University.

† Supported by a grant from the Banting Foundation.

The excretion of various types of corticoids is only a very indirect measure of adrenocortical function and is subject to error due to technical procedure, conjugation of active compounds, and variations in the pathway of metabolism of the steroids. Nevertheless, a number of different types of determinations have been used in an endeavor to elucidate this problem. The biologically active corticoids which have an action on carbohydrate metabolism may be measured by means of the glycogen deposition test (2). The various chemical methods applied to urinary extracts measure certain configurations of the steroid side-chain, but do not distinguish between carbohydrate-active, salt-and-water active, or biologically inactive types of adrenal products. Until recently, no method for the assay of the salt-and-water type of hormone existed. In 1947 Dorfman, Potts and Feil (3) demonstrated that small quantities of desoxycorticosterone acetate caused a reduction in the excretion of sodium-24 in rats. Following this, Deming and Luetscher (4), employing a method based on principles suggested by Dorfman *et al.*, were able to detect significant amounts of sodium-retaining activity in the urine of nephrotic patients. During the diuresis which followed the administration either of cortisone or ACTH, sodium-retaining activity could no longer be demonstrated (5).

Farnsworth and Dupee (6) measured the excretion of formaldehydogenic corticoids in the urine of untreated nephrotic patients and found it to be within the normal range. During treatment with ACTH the excretion of these substances increased markedly in patients who showed an excellent clinical response, but only slightly in others.

CLINICAL MATERIAL AND METHODS

The 14 children on whom the present studies were made, with 1 exception, were hospitalized at the Children's Memorial Hospital. Their ages ranged between 17 months and 12 years. All fulfilled the conventional criteria for diagnosis of the nephrotic syndrome and were edematous at the time of study. None showed persistent hypertension, azotemia, or hematuria, although in most patients transient elevation of the level of serum nonprotein nitrogen had been present and red cells in small numbers were frequently found in the urine. The patients were kept in bed and were weighed daily. Diets usually contained not more than 2 Gm. of salt daily but were otherwise unrestricted. Continuous 24-hour urine collections were attempted in all but the 3 youngest children (M.L., G.N. and D.W.).

ACTH was administered to 10 of the children for periods ranging from six to fourteen days. Frequent measurements of urinary formaldehydogenic corticoid excretion were carried out while the children were receiving the hormone. In 7 cases corticoid determinations were also made during

the few days immediately preceding or following ACTH therapy. Armour ACTH (Lots 32904 and 30902) was used in 11 of the studies, and a Connaught preparation (Lot C-6) in 3. Dosages in milligrams are indicated in Figure 1 and are equivalent to those of the LA-1-A standard.

Assays for the detection of sodium-retaining activity in the urine were carried out on essentially the same group of patients.

In addition to the steroid determinations, daily protein, creatinine and sodium excretions were measured quantitatively. Serum electrolyte, protein and cholesterol concentrations were followed, as were sedimentation rates and total white, differential and absolute eosinophil counts. These, however, are not reported in this paper.

Formaldehydogenic corticoids were determined by a modification of the method of Daughaday, Jaffe and Williams (7). In this laboratory, normal values for children under 12 years of age range from 0.05 mg. to 0.4 mg. per twenty-four hours.

The excretion of urinary sodium-retaining substances was determined by the method of Singer and Venning (8). It is based on the fact that desoxycorticosterone causes retention of sodium in adrenalectomized animals, and is carried out as follows. After acidification, 24-hour specimens of urine are extracted with chloroform. The extracts are refrigerated in a small quantity of absolute alcohol in sealed ampoules, until assay is performed. The test animals are hooded male rats weighing 160–170 grams at the time of adrenalectomy. Two days after adrenalectomy each test animal receives subcutaneous injections of an extract of a twenty-minute urine sample in 1 ml. of absolute alcohol, and 1 ml. of an aqueous solution containing 3.5 mg. of sodium chloride and tracer quantities of radioactive sodium (sodium-24). Each extract is assayed in 6 to 8 animals. At the same time a control group of approximately 8 similarly prepared animals is injected with 1 ml. of absolute alcohol and the radioactive salt solution. Five hours after receiving the radioactive sodium, the animals are killed and the urine removed from the bladder, the urethra having been tied off at the time of the first injection. The mean amount of sodium-24 in the urine of the control animals is considered as 100 per cent excretion. The mean excretion of radioactive sodium in the test animals is expressed as a percentage of that of the control animals. Values under 100 per cent indicate sodium retention. These can be expressed in terms of desoxycorticosterone acetate (DCA) equivalents, based on the effect of this substance in the assay.

RESULTS

Formaldehydogenic corticoids

Results of the urinary excretion studies of formaldehydogenic corticoids in 10 children are presented in Figure 1. In 6 patients, excretion values during the pretreatment period ranged from 0.03 mg. to 0.3 mg. for the 24-hour period and thus fell within normal limits. In response to the administration of ACTH, the output of corticoids increased to more than 1.0 mg. daily in 6 out of 10 patients. In certain children, excretion levels of 2.0 to 3.5 mg. daily were reached. These patients all underwent complete diuresis as a result of therapy. In the 4 remaining patients (A.G., M.L., P.W. and A.C.), excretion values during the first course of ACTH therapy did not exceed 0.5 mg. daily on any occasion, and the ensuing diuresis was

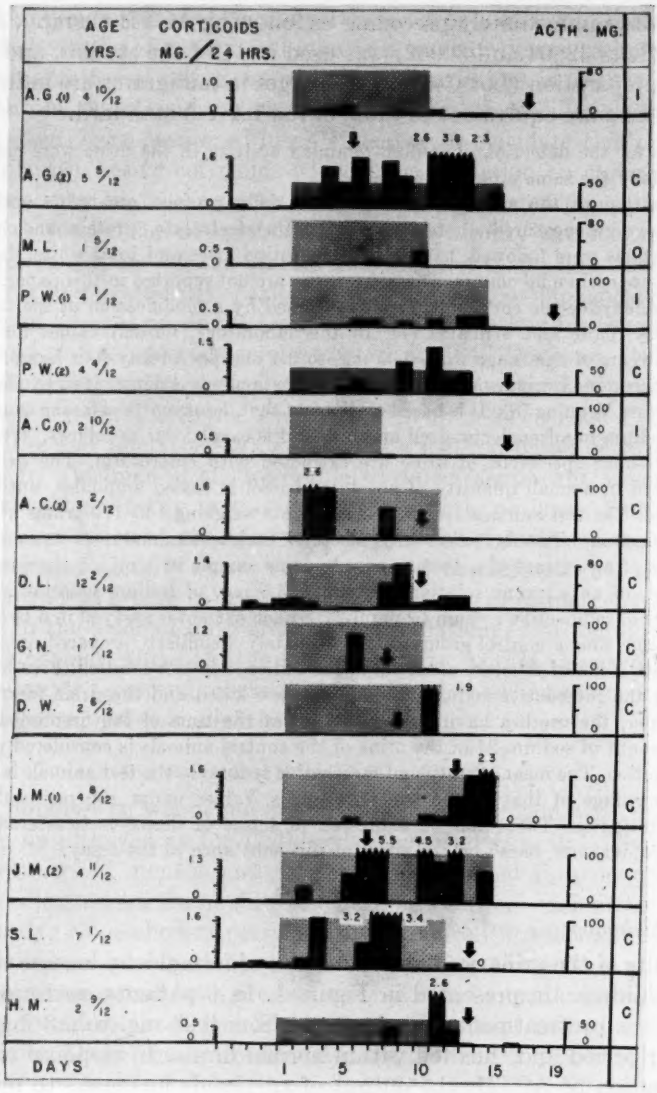

FIG. 1. Response of the urinary formaldehydogenic corticoids to ACTH in nephrosis, and its relation to diuresis. Corticoid excretions (mg./24 hrs.) denoted by dark columns. Duration of ACTH therapy and dose are indicated by shaded areas (light grey indicates Armour ACTH; dark grey, Connaught ACTH). Onset of diuresis is indicated by broad arrow. Completeness (C) and incompleteness (I) of diuresis are shown in extreme right hand column. 0 indicates failure of diuresis.

not satisfactory. Hence a correlation was observed between the level of corticoid excretion in the urine and the diuretic response to ACTH therapy. Those children in whom the urinary corticoid levels failed to show a marked increase with ACTH did not discharge edema, or did so only partially and after a delay of several days. The remaining patients, whose excretions were high at some time during therapy, underwent complete diuresis.

Considerable variation was seen in the rapidity with which the urinary corticoid response to ACTH occurred, as well as in its magnitude. A second course of hormone administered to 4 patients (A.G., P.W., A.C. and J.M.) resulted in increased responsiveness, as indicated by an earlier and more marked increase in corticoid excretion. This improved response

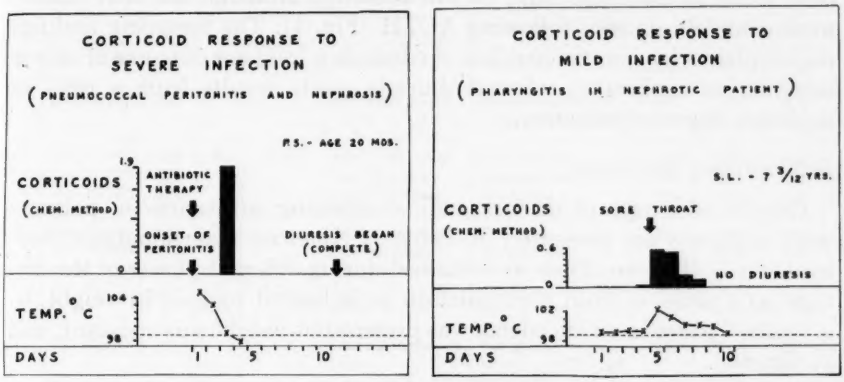

FIGURE 2

was followed by diuresis in the 3 patients who had failed to discharge edema adequately after the previous course of ACTH.

An association also appeared to exist between very active response, as judged by corticoid values, and the early onset of diuresis. Three patients (A.G., A.C. and J.M.), whose urinary corticoid excretions showed a sharp increase soon after the second course of therapy was instituted, began to put out edema fluid in the urine while receiving ACTH. In 2 others (S.L. and D.L.), with comparable corticoid response to ACTH, diuresis started immediately after withdrawal of the hormone.

In all 7 patients in whom measurements of corticoid excretion were made on the day immediately following cessation of therapy, values had decreased sharply to within the normal range. This was true, whether or not diuresis was occurring.

Figure 2 shows the corticoid excretion in 2 children with infection.

One (P.S.) had pneumococcal peritonitis and the other (S.L.) mild pharyngitis. Both children were receiving only antibiotic therapy at the time of assay. The corticoid excretion in P.S. was within the same range as that reached by nephrotic patients who are receiving treatment with ACTH and who subsequently clear their edema. In this patient, diuresis occurred on the eleventh day after the onset of infection. Edema was completely eliminated and the patient has remained free of any sign of the disease, including albuminuria, for thirteen months. The corticoid excretion in the second patient (S.L.) increased suddenly from normal levels to values of 0.6 mg. daily during a minor throat infection. These values are comparable to those obtained in patients who are receiving ACTH but whose subsequent diuretic response is unsatisfactory. It is of interest that this patient failed to discharge fluid on this occasion, although she later underwent complete diuresis following ACTH (Fig. 1). The foregoing findings may explain why spontaneous loss of edema is a frequent outcome of severe infection in nephrotics, whereas diuresis rarely results from a mild or moderate degree of infection.

Salt-retaining substances

Results of assays of urinary sodium-retaining substances in patients with nephrosis are presented in Table 1. Nineteen determinations were made on 11 children. They were studied during different phases of the disease: a) a phase of fluid accumulation as indicated by gain in weight, b) a "static" phase in which edema was present but weight was constant, and c) diuresis.

Assays were made in 9 patients while they were accumulating edema fluid. Eight showed marked sodium-retaining activity in their urinary extracts. In the ninth patient (D.W.), the urinary volume and creatinine level were both very low and the urinary collection was probably incomplete. The sodium-retaining activity shown in this group of children was considerably greater than that observed in normal children (Fig. 3). The average sodium-24 excretion in the test animals was 61.2 per cent of that of the control animals (equivalent to 3.6 μ of desoxycorticosterone acetate (DCA)), whereas the excretion in animals treated with urinary extracts from normal children was 97.1 per cent of that of the control animals. The difference between the two groups is statistically significant (P is <0.01).

Six determinations were made in 4 edematous patients who were in a static state with respect to their edema. Marked sodium-retaining activity was detected on only 1 occasion (in Patient A.C.). Patient J.S. was studied during the phase of fluid accumulation and again during a "static" phase while receiving intravenous ACTH therapy. The marked sodium-

TABLE 1. SODIUM-RETAINING SUBSTANCES IN THE URINE OF PATIENTS WITH THE NEPHROTIC SYNDROME

Subject	Date	Age (yrs.)	Sex	Duration of symptoms	Extent of edema	Clinical status	Urine vol. (ml./24 hrs.)	Urinary Na-24* (% of control)	Approximate DCA equivalent (μ)
A.G.	Nov. 28, 1951	4 1/12	M	6 wks.	Mod. edema & ascites	Gaining wt. slowly; Na output falling.	300	60.2	4.0
	Feb. 17, 1952	5 2/12			Very mild edema	At end of sl. gain in wt. following infection	220	108.5	0
S.L.	Dec. 28, 1951	7	F	6 dys.	Mild edema	Gaining wt. slowly.	263	68.4	3.1
	Jan. 29, 1952	7 1/12			Edema still present	Beginning of salt & water diuresis (1st day after cessation of ACTH)	1400	103.0	0
J.M.	Jan. 12, 1952	4 8/12	M	3 mos.	Mild edema	Gaining wt.	145	56.8	4.3
	Jan. 31, 1952	4 8/12			Edema still present	Beginning of water diuresis (13th day ACTH)	1340	97.1	0.2
A.C.	Sept. 24, 1951	2 10/12	M	3 mos.	Very marked edema	Not gaining wt.	185	54.9	4.6
	Jan. 14, 1952	3 2/12			Very slight edema	Not gaining wt.	360	79.8	2.0
	Jan. 15, 1952	3 3/12			Very slight edema	Not gaining wt.	75	70.1	3.0
P.W.	Sept. 20, 1951	3 9/12	F	11 mos.	Very marked edema	Gaining wt.	200	46.2	5.6
	Jan. 22, 1952	4			Moderate edema	Not gaining wt.	325	84.8	1.5
J.S.	Apr. 14, 1952	4 6/12	M	3 yrs.	Edema of face and ascites	Accumulating fluid rapidly.	215	56.2	4.3
	Apr. 20, 1952	4 7/12			Edema and ascites still present	Not gaining or losing wt. on i.v. ACTH (high fluid intake)	910	100.0	0
D.W.	Jan. 3, 1952	2 6/12	M	3 wks.	Mod. edema & ascites	Gaining wt. slowly	79	97.1	0.2
R.M.	Jan. 17, 1952	2	M	1 mo.	Moderate edema	Not gaining wt.	165	90.1	1.0
J.P.S.	Apr. 23, 1952	7	M	12 dys.	Moderate edema	Gaining wt. rapidly	176	59.0	4.1
H.M.	May 13, 1952	2 6/12	F	2 wks.	Fairly marked edema	Rapidly accumulating fluid.	105	57.1	4.2
	May 27, 1952	2 6/12			Edema still present	Beginning of water diuresis (1st day after ACTH)	1025	95.9	0.3
A.D.L.	June 3, 1952	1 6/12	F	3.5 mos.	Marked edema	Rapidly accumulating fluid	190	49.7	5.0

* Radioactive sodium (Na-24) excretion of control animals was considered as 100 per cent. Values under 100 per cent indicate sodium retention.

retaining effect observed in this patient prior to ACTH therapy (test animals excreted 56.2 per cent as much sodium-24 as the control animals) was no longer evident on the sixth day of ACTH administration.

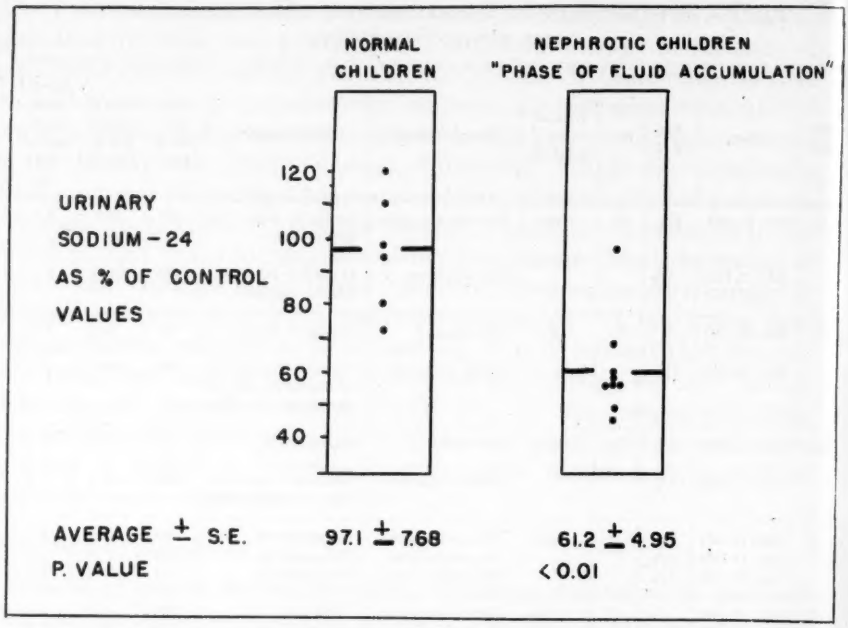

FIG. 3. Effects of urinary extracts obtained from normal children, and from patients with nephrosis during the phase of "fluid accumulation," on the excretion of sodium-24 in rats. An extract of a twenty-minute sample of urine was used for each rat. Sodium-24 excretion of control animals was considered as 100 per cent. Values under 100 per cent indicate sodium retention. (S.E. = standard error.)

Three patients (S.L., J.M. and H.M.) were studied, both during the phase of fluid accumulation and during diuresis which occurred on ACTH therapy or immediately following cessation of ACTH administration (Fig. 4). The marked sodium-retaining effect observed when patients were accumulating fluid (test animals excreted 60.8 per cent as much sodium-24 as the control animals) was no longer evident during the phase of diuresis.

DISCUSSION

Results of measurement of urinary formaldehydogenic corticoids in nephrotic children suggest that a significant increase in the excretion of these substances must occur in response to ACTH therapy, if complete diuresis is to follow. The present study also indicates that not only is there great variation in the urinary corticoid response to ACTH in different nephrotic patients, but also that the corticoid response of a patient to subsequent courses of ACTH therapy may be increased. These findings confirm the earlier observations of Farnsworth and Dupee.

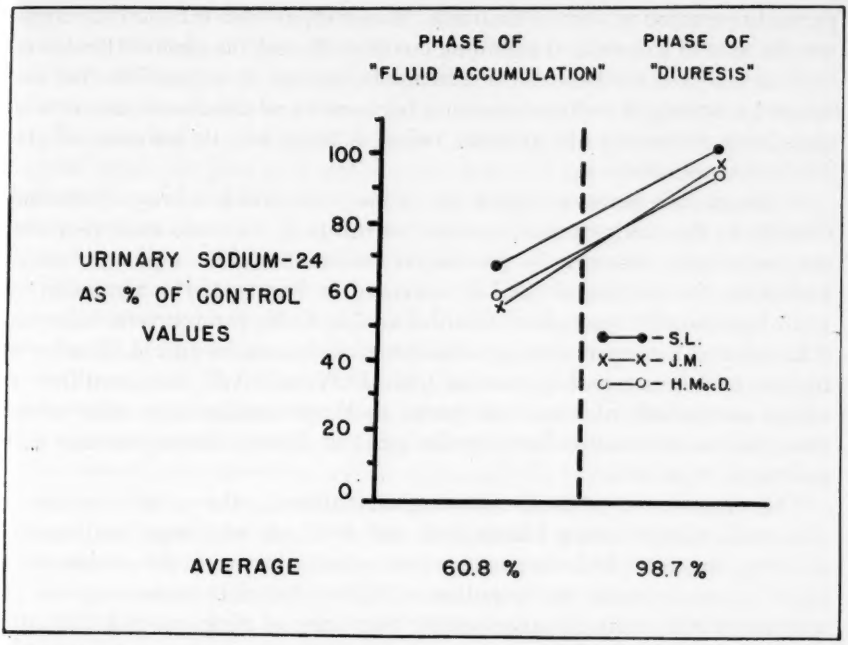

FIG. 4. Sodium-retaining activity in the urine of children with nephrosis, before and after diuresis, as indicated by the excretion of sodium-24 in rats. An extract of a twenty-minute sample was used for each rat. Sodium-24 excretion of control animals was considered as 100 per cent. Values under 100 per cent indicate sodium retention.

The reason for the variability of the urinary corticoid response to adrenal stimulation by ACTH is not clear. It is possible that some of the injected ACTH may be lost with other proteins in the urine. Again, it may be that part of the injected ACTH is destroyed peripherally before it can cause adrenal stimulation. During the study it was noted that patients with the lowest corticoid excretion values during ACTH therapy had massive edema. Certain preparations of ACTH are thought to be inactivated by tissues, because they are many times more efficacious by the intravenous than by the intramuscular route. In the presence of marked edema, absorption might well be delayed, and the prolonged contact of the injected hormone with the tissue cells would favor its inactivation. There is a possibility that the improvement in response during repeated courses, as seen in our study, may be due to the fact that less edema was present at the time of the second course of therapy. Finally, in patients with nephrosis, adrenal function may in some way be altered or impaired. In this disease, corticoids, as determined by chemical methods, are ap-

parently excreted in normal amounts. However, as such estimations measure the sum of the sodium-retaining compounds and the glucocorticoids as well as inactive metabolites of adrenal hormones, it is possible that increased amounts of sodium-retaining hormones and decreased amounts of glucogenic corticoids are actually being secreted by the adrenals of the nephrotic patients.

Although adequate increases in urinary corticoids always preceded diuresis in the cases studied, marked elevation in corticoid excretion was not necessarily followed by prolonged remission of the nephrotic state. Following the courses of ACTH indicated in Figure 1, the remission in D.L. has lasted twenty-four months and in G.N. for fourteen months. S.L. suffered a relapse after a remission of nine months and H.M. after a remission of four months, whereas J.M., D.W. and A.G. remained free of edema for periods of from two weeks to three months only. Moreover, there was no association between the onset of diuresis during therapy and prolonged remission.

The detection of sodium-retaining substances in the urine of patients who were accumulating edema, but not in those who were undergoing diuresis, suggests that there may be a relation between the excretion of these substances and the retention of fluid. That this factor may be of adrenocortical origin is suggested by the work of Simpson and Tait (9) who found, both in adrenal extracts and in urine, a substance which had a sodium-retaining effect about twenty times that of DCA. The present findings confirm the work of Deming and Luetscher who found increased amounts of DCA-like substances in the urine of patients with nephrosis.

Should an imbalance in the secretion of hormones of the adrenal gland, with predominance of salt-retaining hormone secretion, be of importance in the production of edema in nephrosis, reversal of the imbalance might tend to bring about diuresis. Such a reversal may occur when ACTH is administered, for its effect is considered to be predominantly a glucocorticoid one. In the present study, untreated nephrotic children were observed to excrete abnormally high quantities of sodium-retaining substances and normal amounts of formaldehydogenic corticoids. During ACTH administration the excretion of formaldehydogenic corticoids was noticeably increased in those patients who responded with diuresis. As previously noted by Luetscher, ACTH therapy also resulted in a decrease in the excretion of salt-retaining factor in patients (J.M. and J.S., Table 1) who were studied immediately before and during therapy. These observations support the view that, during its administration, ACTH may reverse the hormonal imbalance which appears to be present in the untreated disease. Withdrawal of ACTH was followed by a sharp decrease in the excretion of

formaldehydogenic corticoids to very low levels, and by disappearance of sodium-retaining activity. It is of interest that it is during this period of relative adrenal inactivity that the onset of diuresis frequently occurs.

It is possible that the adrenal hormone imbalance suggested by these studies may reflect abnormal pituitary activity. In the presence of augmented posterior pituitary antidiuretic activity, for example, one might expect the compensatory release of additional amounts of adrenal salt-retaining hormones. Or the pituitary overactivity might be of a different type. Selye (10) found that crude anterior pituitary extracts had mineralocorticoid effects when injected into sensitized animals. He also showed that growth hormone produces similar effects (11). As part of a study of the effect of the intravenous administration of growth hormone in man (12), it was found that the sodium-retaining substances in the urine of 2 normal adults increased significantly following administration of the hormone (13). It is conceivable that the protein depletion of the nephrotic state may provide a stimulus for increased release of an anabolic hormone such as growth hormone. This, in turn, could stimulate mineralocorticoid release from the adrenal and result in the retention of salt and water. Study of growth hormone production in children with nephrosis would be of interest.

The present study indicates that there is a disturbance in adrenal function in nephrosis. It is possible that this is secondary to a disturbance in the pituitary, either posterior or anterior, and that these hormonal abnormalities may in part be responsible for the edema.

SUMMARY

Urinary formaldehydogenic corticoid excretions of nephrotic children receiving ACTH by intramuscular injection increased markedly in patients who responded with complete and prompt clearing of edema. Delayed or incomplete diuresis followed coursed in which no great increase in corticoid excretion was observed. Repeated courses of the hormone resulted in increased responsiveness, as judged by corticoid excretion.

Corticoid excretion in a nephrotic child at the onset of pneumococcal peritonitis was comparable to that seen when response to ACTH is adequate.

The urinary excretion of a salt-retaining factor was detected in 8 out of 9 patients who were accumulating edema fluid. In the 3 patients on whom the assay was carried out during diuresis, sodium-retaining activity was not present. Marked sodium-retaining activity was demonstrable in only 1 out of 6 determinations on chronically edematous patients who were no longer gaining weight.

Adrenal hormone imbalance occurs in nephrosis and may play a part in the formation of edema.

Acknowledgments

The authors are indebted to Dr. Eleanor Venning and Dr. J. S. L. Browne for advice in the preparation of this paper.

Technical assistance was contributed by Mr. H. Ramus and Miss H. Stone. Miss J. Belcourt performed the formaldehydogenic corticoid determinations.

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STUDIES ON ADRENOCORTICAL FUNCTION IN RELATION TO THE NEPHROTIC SYNDROME*

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OBSERVATIONS on the stimulation of the adrenal cortex by ACTH (Armour) in the nephrotic syndrome were reported in October, 1949 (1). Massive release of edema fluid with a reduction in the level of serum cholesterol and an increase in the level of serum proteins, during or immediately following the experimental period, were the major findings. Further experiences with ACTH in nephrosis were subsequently reported (2–7). Although the majority of remissions so induced were incomplete, the observations showed the importance of investigation directed toward 1) the pituitary-adrenal complex as a contributory factor in the pathogenesis of nephrosis, or 2) the nephrotic syndrome as a disturbance peculiarly adapted to display the actions of adrenocorticotropin. The studies which are presented here were therefore organized for the purpose of determining or assaying 1) the condition of the adrenals before treatment, 2) the degree of activation obtained, 3) the correlation of glandular activity with clinical results, and 4) the effect of repeated stimulation.

METHODS

The methods employed in this study were standard procedures. The flame photometer was used for quantitative determinations of sodium and potassium. Nitrogen intake was determined by serving articles of diet singly and weighing the plates before and after meals; the nitrogen content was then calculated from tables spot-checked and approved by the Department of Biochemistry. Fluid intake was measured by weight rather than by volume. Urine collections were kept refrigerated. Urinary protein was determined on filtered or centrifuged urine by precipitation with Tsuchiya's reagent.

Corticoid determinations were performed by the periodic acid method after acid hydrolysis. Determinations were also made upon specimens pretreated with beta-glucuronidase. For these assays 25-ml. aliquots were employed; the enzyme (300 mg.) was added to each sample, and incubation was carried on at a pH of 4.5 for forty-eight hours. After incubation, the pH was reduced to 1.0, and extraction was started. The values thus derived resulted in findings for normal individuals which were somewhat

Received for publication September 11, 1952.

* This study was aided by a grant from the U. S. Public Health, Division of Research Grants and Fellowships.

lower than those quoted by other research groups, notably Corcoran and co-workers, but reproducibility was satisfactory and the trend of corticoid excretion during treatment was consistent.

The 17-ketosteroid determinations were performed on the ketonic fraction, using aliquots of 50 ml. Extraction was carried out with ether, and Girard-T reagent was employed.

CLINICAL MATERIAL

The series consisted of 15 patients ranging in age from 5 to 58 years. Three of this series had no detectable edema but had marked proteinuria, hypoproteinemia and hypercholesterolemia. No attempt was made to differentiate between "lipoid nephrosis" and nephrosis appearing as a sequela to acute glomerulonephritis. Twelve patients received two or more courses of ACTH. The case histories which follow are brief abstracts of the entire course. The tables are condensed to cover a single episode, hence values are sometimes quoted in the text which are not found in the tables.

Treatment with adrenocorticotropin was administered by the intramuscular route in divided doses, except for several instances in which medication was given intravenously. In such cases, specific reference is made in the tables to the intravenous route.

T.K., a 4-year-old male, had been well until May 1949, at which time vomiting and periorbital swelling developed. Urinalysis at that time showed massive proteinuria. Diagnosis of a kidney disorder was made, and the patient was given a low-salt diet. Notwithstanding this management, ascites and generalized edema became marked. Abdominal paracentesis was performed in September. The patient was admitted to Passavant Memorial Hospital in October 1950, where he was treated with ACTH (Table 1).

Course: Two courses of ACTH were given, as indicated in Table 1, the second of which was associated with diuresis.

A serum urea nitrogen concentration of 31 mg./100 ml. in the control period rose to 56 mg./100 ml. during ACTH therapy and returned to the control level at the time of discharge. The blood cholesterol level fell from 1291 mg./100 ml. on admission to 740 mg./100 ml. at the time of discharge. The concentration of serum proteins increased slowly. The urinary protein increased during ACTH administration. The steroid excretion was low during the first course, but increased during the second.

Classification—partial remission.

In January 1951, the patient was readmitted to the hospital. The general clinical condition was considerably better than at the time of the first admission, but the level of the serum cholesterol had increased somewhat and that of the serum proteins had decreased. The patient was discharged after protracted treatment on May 11, 1951, and received no further treatment. Chemical determinations on a blood sample taken on January 26, 1953 are included in Table 1.

R.F., a 5-year-old male (Table 2), entered Passavant Memorial Hospital March 31, 1950, with edema of three months' duration. The past history included virus pneumonia

TABLE 1. T.K.

Date	*Serum*						*Urine*							Body wt. (Kg.)	ACTH (I.U. per day)
	Urea N (mg./100 ml.)	Creat. (mg./100 ml.)	P (mg./100 ml.)	Chol. (mg./100 ml.)	Tot. prot. (Gm./100 ml.)	Alb. (Gm./100 ml.)	Vol. (ml./24 hrs.)	Na (mEq./24 hrs.)	K (mEq./24 hrs.)	Cl (mEq./24 hrs.)	17-KS (mg./24 hrs.)	Cort. S.* (mg./24 hrs.)	Prot. (Gm./24 hrs.)		
1950															
Oct. 25	31	2.5	4.7	1,291	2.7	0.5	395	2	21	15	1.5	0.9	3.4	20.7	
Nov. 1							750	9	37	27	1.9	6.5	6.5	21.2	50 (Oct. 31–Nov. 4)
3							1,030	12	59	44	2.8	11.9	7.4	22.9	
5	51	2.1	4.2	956	3.3	0.5	1,515	65	81	113	3.8	8.7	5.4	23.4	
7							1,230	33	76	77	3.4	6.6	4.4	21.2	
9							1,030	28	71	71	4.3	6.1	2.9	21.8	
11							980	11	67	46	3.5	15.1	1.6	22.4	
13	53	2.3	4.2	832	4.5	0.2	1,385	53	70	88	5.7	15.6	6.9		50 (Nov. 13–23)
15							1,700	70	75	105	9.1	12.5	7.3	21.2	
17							1,895	76	91	131	11.1	17.1	6.5	21.4	
19	56	2.2	5.9	804	4.6	0.5	1,622	46	79	88	13.3	22.1	7.6	20.2	
21							1,600	46	54	65	18.2	19.3	3.4	20.6	
23							1,540	77	68	89	21.4	8.6	5.0	19.3	
25							1,440	40	69	62	16.2	9.4	7.2	18.0	
27	38	1.5	3.2	740	3.7	0.9	1,455	56	72	83	16.2	5.1	1.3	18.9	
Dec. 5	32	1.7	4.5		4.0	0.9									
1953															
Jan. 26	23	1.3	3.5	315	6.5	4.0							0.5		

* Glucuronidase method.

TABLE 2. R.F.

Date	*Serum*						*Urine*							Body wt. (Kg.)	ACTH (I.U. per day)
	Urea N (mg./100 ml.)	Creat. (mg./100 ml.)	P (mg./100 ml.)	Chol. (mg./100 ml.)	Tot. prot. (Gm./100 ml.)	Alb. (Gm./100 ml.)	Vol. (ml./24 hrs.)	Na (mEq./24 hrs.)	K (mEq./24 hrs.)	Cl (mEq./24 hrs.)	17-KS (mg./24 hrs.)	Cort. S. (mg./24 hrs.)	Prot. (Gm./24 hrs.)		
1950															
Apr. 4	55	1.9	5.6	1,670	2.6	0.9								26.0	75 (Apr. 5–10)
10	51	1.1	6.5	2,033	3.0	1.2								26.2	
15							275	2	16	7	1.1	0.1	4.3	26.2	
17	24	1.0	4.3	1,328	3.2	1.0	285								25 (Apr. 17–)
19							265	1	13	4	2.9	0.9	7.1	27.9	50 (Apr. 18–29)
21							386	2	35	10	3.6	0.6	4.1	28.3	
23							350	2	34	14	6.6	0.6	2.2	28.5	
25	16	1.3	3.6	1,590			414	2	41	28	6.3	0.2	5.6	28.9	
27							719	12	75	71		0.1	8.3	29.0	
29							1,292	148	49	174	7.2	0.4	1.8	28.9	
May 1	8	0.9	4.6	885	4.8	1.8	3,677	412	66	414	6.8	0.4	1.7	24.1	
3							2,098	284	88	198	5.7	0.3	2.4	19.1	
9	9	0.8	4.1	789	5.4	2.4	915	40	57	57	2.4	0.4	2.9	19.4	

at 2 years, chicken pox at 3 years and scarlet fever in January 1949. Recovery appeared to be complete and the patient was well until July 1949, when periorbital edema was noted each morning for a period of two weeks. In October, he had acute tonsilitis, which was treated with penicillin. Several days later the urine was found to contain albumin and casts. In January 1950, the edema recurred, involving the face and lower extremities; it was accompanied by rapidly increasing ascites. Physical examination after admission to the hospital revealed no significant findings beyond those already noted. The systolic blood pressure was 130 and the diastolic, 90 mm. of Hg.

The patient was given two courses of ACTH (Table 2) the second of which was followed by a massive diuresis. He was discharged on May 12. A return of the edema with a rise in the serum cholesterol level as determined in the local hospital led to the patient's readmission to Passavant on June 8.

Course: The first course of ACTH failed to produce diuresis. The second resulted in a weight loss of approximately 10 Kg.

The initial serum urea nitrogen level of 55 mg./100 ml. did not change significantly during or immediately after the first course, but decreased to 24 mg. per 100 ml. in the control determination prior to the second course. Thereafter, all values were within the range of normal. The serum cholesterol concentration increased somewhat with each course but declined markedly during the one to two weeks of observation following therapy. The level of the serum proteins rose steadily. The urinary protein loss increased during treatment and decreased thereafter. The corticosteroid output was low throughout.

Classification—partial remission.

K.W.D., a 7-year-old boy (Table 3), had been in good health until his family left their home in Western Canada in October 1949, and moved to Chicago. Since that time he had frequent respiratory infections. In January 1950, he had chicken pox and otitis media which subsided with penicillin. Swelling of the face was noted by his mother in April; this rapidly developed into a generalized anasarca. He was treated at Augustana Hospital until admitted to Passavant on May 6, 1950. The child was grossly edematous at this time. There was a variable, low-grade elevation of temperature. The blood pressure was 100/60. The urine was loaded with casts and contained a few erythrocytes and leucocytes. Improvement during the first course of ACTH was so gratifying that the course was prolonged in diminishing doses until proteinuria and edema had disappeared and the levels of the chemical constituents in the blood were restored to normal. Several weeks later an acute otitis media developed, which was soon associated with an exacerbation of his nephrosis. He was readmitted to the hospital and given a second course of treatment similar to the first. The data pertaining to both admissions are given in Table 3.

Classification—complete remission followed by relapse and a second remission. Follow-up reports indicated that there was no further relapse.

L.T., a 7-year-old female (Table 4), was admitted to the hospital in July 1950, with a known nephritis of two months' duration. The onset had been characterized by the appearance of periorbital edema, which led to the finding of protein in the urine. She had entered Michael Reese Hospital at that time, and a marked decrease in the level of serum protein, an increase in serum cholesterol and moderate elevation of blood pressure were found. She was discharged with a diagnosis of nephrosis. Upon admission to Passavant, the patient was found to be a thin, somewhat sallow child without notable physical

TABLE 3. K.W.D.

Date	Serum						Urine							Body wt. (Kg.)	ACTH (I.U. per day)
	Urea N (mg./100 ml.)	Creat. (mg./100 ml.)	P (mg./100 ml.)	Chol. (mg./100 ml.)	Tot. prot. (Gm./100 ml.)	Alb. (Gm./100 ml.)	Vol (ml./24 hrs.)	Na (mEq./24 hrs.)	K (mEq./24 hrs.)	Cl (mEq./24 hrs.)	17-KS (mg./24 hrs.)	Cort. S.* (mg./24 hrs.)	Prot. (Gm./24 hrs.)		
1950															
May 8				998	4.3	1.2	455	2		45	1.88	0.3	1.9	31.1	
11							360	8		39	2.7	1.4	0.6	31.2	50 (May 11–22)
13							382	9		29	4.0	1.2	1.7	31.9	
15							1,522	212		209	5.1	4.7	1.2	31.5	
17				745	3.9	1.5	2,390	339		285	5.9	5.8	0.7	29.5	
19							1,655	198		169	17.3	7.7	0.4	25.5	
21							1,550	164		155	15.4	4.6	0.1	23.7	
23							1,365	153		156	8.5	0.6	0.0	23.1	25 (May 23–26)
25				578	5.9	3.3	1,550	70		87	3.5	1.3	0.0	22.8	
June 5				324	6.4	4.3									
12				218	6.6	4.3									
19				333	5.2	2.7									
24							515	32		36	2.9	1.6*	1.5	25.4	50 (June 24–July 8)
26				404	4.9	2.6	475	43		47	4.7	1.9	0.6	25.7	
28							752	60		57	5.7	2.9	2.2	24.7	
30							870	82		65	7.0	3.3	0.0	24.5	
July 2							865	54		57	5.4	3.4	0.6	24.3	
4				481	5.7	3.3	1,190	59		77	10.2	26.0	0.0	23.9	
10				265	6.0	3.5									

* Glucuronidase method.

TABLE 4. L.T.

Date	Serum						Urine							ACTH (I.U. per day)
	Urea N (mg./100 ml.)	Creat. (mg./100 ml.)	P (mg./100 ml.)	Chol. (mg./100 ml.)	Tot. prot. (Gm./100 ml.)	Alb. (Gm./100 ml.)	Vol. (mg./24 hrs.)	Na (mEq./24 hrs.)	K (mEq./24 hrs.)	Cl (mEq./24 hrs.)	17-KS (mg./24 hrs.)	Cort. S (mg./24 hrs.)	Prot. (Gm./24 hrs.)	
1950														
July 6				531	4.8	1.5	385	12		36	2.4	0.5	0.6	
8							320	10		29	1.9	1.0	0.8	50 (July 8–19)
10				460	4.6	1.6	365	4		12	1.9	2.0	0.8	
12							425	8		22	3.9	3.0	1.2	
14							825	42		65	6.3	5.8	0.1	
16							920	65		59	9.6	20.2	Tr.	
18				477	5.0	2.0	1,490	74		89	9.8	4.9	0.0	
20							1,065	51		74	7.6	8.9	0.0	25 (July 20–21)
22							1,082	37		70	12.1	8.4	0.0	
24				319	5.5	2.6	1,035	70		85	16.7	4.6	0.0	
26							1,015	38		56	7.8		0.0	
28							570	18		39	2.5	0.9	0.0	
31				273	5.5	2.9								
Aug. 8				319	5.9	3.1								
22				197	6.4	3.5								

abnormality. The blood pressure was 104/72. She was treated with ACTH according to the schedule indicated in Table 4.

Course: Since there was no gross edema, values for body weight have been omitted from the table. The moderate retention of salt which occurred during the first days on ACTH was reversed during continued administration, as shown in the sodium excretion column.

All serum urea nitrogen values remained within normal limits and therefore have been omitted from the table. The serum cholesterol level declined consistently. The values for serum proteins increased steadily during and after treatment. The urinary protein disappeared during the course. Corticosteroid excretion was high.

Classification—complete remission.

D.D., a 7-year-old boy (Table 5), was admitted to Passavant Memorial Hospital on January 7, 1950, complaining of swelling of the dependent parts, abdomen and face. He had been well until May 28, 1949, when tonsillectomy had been performed. Eight weeks later, puffiness around the eyes was noted, along with edema, ascites, diarrhea and sore throat. The patient was brought into Bobs Roberts Hospital, University of Chicago, where he was treated for five months.

Massive ascites necessitated abdominal paracentesis on several occasions. The patient was admitted to Passavant Hospital in January 1950.

Course: Figures for body weight have been omitted from Table 5 because paracentesis, performed prior to the course of ACTH, resulted in continued leakage of fluid. The values for urine sodium and chloride, however, show the diuresis which followed the course.

The serum urea level rose somewhat during treatment and decreased during the following week. The concentration of serum cholesterol dropped notably following the course, and that of the serum proteins increased slightly. The urinary protein decreased significantly following ACTH. The corticoid excretion was moderately low.

Classification—partial remission. Follow-up chemical determinations on blood and urine performed January 8, 1951, gave normal results.

M.L.M., a 10-year-old girl (Table 6), had been well until February 1944, at which time she had had a cold which had been treated with a sulfa preparation. This was followed by the appearance of hematuria, which was believed by her parents to have cleared up, but in 1947 hematuria and proteinuria were again noted, along with a puffiness of the face. In May 1948, a streptococcal infection of the throat was followed by generalized anasarca, which persisted in varying degree until her admission to Passavant in May 1950. Physical examination at that time revealed only puffiness and pallor without other abnormalities. The blood pressure was 140/100 but decreased promptly to 130/90. The urine was loaded with casts but showed only occasional cells.

The data collected during hospital admission are presented in Table 6.

Course: Edema was only moderate on admission, and a diuresis of 3 Kg. after the third course served to render the patient edema-free.

The control serum urea nitrogen concentration was 29 mg. per 100 ml.; it rose during treatment and then fell to a level of 17 mg. per 100 ml. after the course. The serum cholesterol concentration decreased from 840 mg. to 507 mg. per 100 ml. after the first course. At the time of discharge the levels of serum proteins were somewhat higher than on admission. An increase in urinary protein during each course of ACTH subsequently subsided to approximately control levels. The steroid excretion was low at all times.

Classification—no remission. Follow-up studies showed no noticeable re-formation of

TABLE 5. D.D.

Date	Serum						Urine							N intake (Gm./24 hrs.)	ACTH (I.U. per day)
	Urea N (mg./100 ml.)	Creat. (mg./100 ml.)	P (mg./100 ml.)	Chol. (mg./100 ml.)	Tot. prot. (Gm./100 ml.)	Alb. (Gm./100 ml.)	Vol. (ml./24 hrs.)	Na (mEq./24 hrs.)	Cl (mEq./24 hrs.)	17-KS (mg./24 hrs.)	Cort. S (mg./24 hrs.)	Prot. (Gm./24 hrs.)	N (Gm./24 hrs.)		
1950															
Jan. 10	21	1.3	4.5	1,380	3.7	0.3	285	1	9		0.3	4.6	4.0	5.4	
12							265	1	11		0.4	5.9	3.8	3.7	
13							220	1	8		0.6	3.0	2.9	6.1	50 (Jan. 13–)
15							250	1	7		1.6	5.6	4.5	6.1	75 (Jan. 14–18)
17	34	1.6	3.3	1,455	3.8	0.3	260	1	10		—	4.5	4.8	6.6	
19							795	8	57		0.5	1.6	5.4	6.9	
21							1,970	74	147		0.3	1.3	4.7	—	
23							1,430	88	105			1.3	3.3	6.9	
25	19	1.3	4.1	625	4.4	0.9	740	52	34			1.5	2.4	7.7	
27							620	54	38			1.0	1.8	7.1	

TABLE 6. M.L.M.

Date	Serum						Urine							Body wt. (Kg.)	ACTH (I.U. per day)
	Urea N (mg./100 ml.)	Creat. (mg./100 ml.)	P (mg./100 ml.)	Chol. (mg./100 ml.)	Tot. prot. (Gm./100 ml.)	Alb. (Gm./100 ml.)	Vol. (ml./24 hrs.)	Na (mEq./24 hrs.)	K (mEq./24 hrs.)	Cl (mEq./24 hrs.)	17-KS (mg./24 hrs.)	Cort. S* (mg./24 hrs.)	Prot. (Gm./24 hrs.)		
1950															
Sept. 1	29	1.9	8.6		4.2	1.3	1,220	14	61	28	5.9	2.8	6.9	32.1	75 (Sept. 3–13)
3							1,000	5	54	14	5.8	8.2	8.2	31.5	
5							850	4	41	13	5.3	3.6	8.3	32.5	
7							1,257	6	56	28	8.7	6.9	8.9	32.5	
9							1,107	2	12	13	8.9	3.6		32.9	
11	39	1.5	6.9	840	4.4	1.1	1,155	7	45	10	9.2	5.6		33.1	
13							1,217	48	51	49	4.3	2.9	6.1	33.3	
15							1,130	44	48	41	3.7	2.1	6.3	33.3	
17							1,397	53	53	51	3.4	1.9	6.0	32.1	25 (Sept. 20–)
19	17	1.5	8.8	507	4.7	1.3	1,179	26	21	41	3.3	4.1	5.5	31.3	75 (Sept. 21–29)
21							1,250	11	71	23	3.4	9.1	5.4	31.5	
23							1,068	6	62	22	3.6	3.0	5.3	32.1	
25							880	23	55	20	4.3	2.3	7.9	32.5	
27	36	1.8	6.9		4.7	1.5	890	3	48	30	9.9	1.2	7.7	32.9	
29							1,190	14	59	27	10.6	0.8	6.8	33.3	
Oct. 1	31	1.6	7.5		4.8	1.6	1,210	17	60	33	5.7	1.8	5.2	32.9	

* Glucuronidase method.

edema. There was a significant increase in the values for serum proteins, urinary protein and serum cholesterol. These improvements, however, were associated with a slow but steady increase in the level of serum creatinine which, when calculated with the urinary creatinine excretion, indicated a diminution in the clearance of endogenous creatinine. These findings led to the impression that the nephrotic syndrome had progressed to the stage of chronic nephritis with renal decompensation.

D.H., a 13-year-old male (Table 7), entered Passavant Memorial Hospital on September 2, 1950, complaining of swelling of the ankles and face of several months' duration. The past history was essentially noncontributory, except for frequent sore throats associated with low-grade fever. In December 1949, urine analysis had revealed albumin (1 plus). On June 7, 1950, discovery of swelling of the ankles led to an examination by the family doctor, who made a diagnosis of "nephritic nephrosis," and started the boy on a high-protein, low-salt diet, with limited exercise. Upon admission to Passavant in September, the blood pressure was found to be 146/84; protein, erythrocytes and leucocytes were present in the urine. The blood erythrocyte count was 3,930,000, the leucocyte count 8,250, the hemoglobin 10.3 grams. The patient was given a course of ACTH according to the schedule indicated in Table 7. He was discharged on September 27 after a satisfactory diuresis and readmitted on October 24, for a second course of treatment.

Course: A complete diuresis of 4.8 Kg. was initiated during the course of treatment. The control serum urea nitrogen level of 24 mg. per 100 ml. rose during treatment and then declined to 15 mg. per 100 ml. several weeks after the conclusion of the course. There was a decrease in the serum cholesterol concentration. The level of serum proteins rose and the urinary protein diminished. The steroid excretion was low during the first course, but increased during the second.

Classification—partial remission. A follow-up examination at six months showed no further edema formation, a slight reduction in level of serum proteins, and maintenance of the reduction in urinary protein excretion.

G.H., a 14-year-old female, was admitted to Passavant Memorial Hospital in April 1950, complaining of pallor and fatigue, with known elevation of blood pressure and albumin in the urine. Birth had been by Caesarian section because of the development of toxemia of pregnancy in the mother. At 6 months of age, a diagnosis of enlarged thymus had been made. At 3 years, the patient had whooping cough of unusual severity, which lasted for nine months; at 7 years, measles and chicken pox. In 1945, she had "bronchitis and asthma" and was given various antihistamines for a period of two years. In July 1949, the urine was found to contain protein, pus and red cells, which failed to disappear with antibiotic treatment. The blood pressure on admission was 170/105. After four days at bed rest it was 140/90. There was no gross edema and no abnormal physical finding. She was given two courses of ACTH as indicated in Table 8.

Course: Moderate fluctuations of body weight occurred during and following each course of ACTH. Sodium excretion was only slightly affected by the first course, much more so by the second.

The serum urea nitrogen concentration increased substantially during the first course. During the second, the rise was hardly significant and the subsequent decline resulted in a value below that of the control. The serum cholesterol level declined, and that of the serum proteins increased moderately. The urinary protein changes were not significant. Excretion of corticosteroids was low during the first course but notably higher during the second.

Classification—partial remission.

TABLE 7. D.H.

Date	*Serum*						*Urine*							Body wt. (Kg.)	ACTH (I.U. per day)
	Urea N (mg./ 100 ml.)	Creat. (mg./ 100 ml.)	P (mg./ 100 ml.)	Chol. (mg./ 100 ml.)	Tot. prot. (Gm./ 100 ml.)	Alb. (Gm./ 100 ml.)	Vol. (ml./ 24 hrs.)	Na (mEq./ 24 hrs.)	K (mEq./ 24 hrs.)	Cl (mEq./ 24 hrs.)	17-KS (mg./ 24 hrs.)	Cort. S* (mg./ 24 hrs.)	Prot. (Gm./ 24 hrs.)		
1950															
Sept. 4							1,780	114	60	110	8.5	2.8	3.6	60.9	
5	24	1.2		380	5.7	2.4	2,240	116	85	107	8.1	3.5	5.0	60.4	
7							1,447	36	87	46	8.7	4.0		59.9	100 (Sept. 7–14)
9							2,012	6	87	23	11.2	6.6		59.9	
11							2,175	13	74	20	4.4	6.5		59.9	
13	27	1.3		—	5.6	2.4	1,980	30	71	33	16.5	4.4	4.0	58.6	75 (Sept. 14–18)
15							2,067	25	83	33	8.3	5.3	5.1	58.6	
17							2,225	13	94	24	20.9	6.0	5.6	58.8	
19	25	1.0			5.6	2.4	3,497	105	56	89	15.9	4.3	5.0	58.7	
21							2,355	94	71	93	9.4	0.6	3.4	57.5	
23							2,445	44	106	55	9.3	1.3	1.8	56.1	
25	32	1.3			5.6	2.5	2,322	14	51	35	3.3	4.5	3.3	55.6	
Oct. 10	15	1.5		215	5.3	3.0									
30	31	1.3			6.8	3.0									100 (Oct. 26–Nov. 7)
Nov. 6	32			225	6.0	2.8									
13	26			261	6.0	3.5									
21	16	1.1			6.5	3.5									

* Glucuronidase method.

TABLE 8. G.H.

Date	*Serum*						*Urine*							N intake (Gm./ 24 hrs.)	ACTH (I.U. per day)
	Urea N (mg./ 100 ml.)	Creat. (mg./ 100 ml.)	P (mg./ 100 ml.)	Chol. (mg / 100 ml.)	Tot. prot. (Gm./ 100 ml.)	Alb. (Gm./ 100 ml.)	Vol. (ml./ 24 hrs.)	Na (mEq./ 24 hrs.)	Cl (mEq./ 24 hrs.)	17-KS (mg./ 24 hrs.)	Cort. S (mg./ 24 hrs.)	Prot. (Gm./ 24 hrs.)	N (Gm./ 24 hrs.)		
1950															
Apr. 25	24	1.3	5.1	560	4.5	2.5	1,120	75	76	6.2	0.2	3.1	8.8	12.5	
28							960	41	46	9.9	0.2	2.9	11.0	18.5	70 (Apr. 28–30)
30							780	36	45	9.9	0.2	2.5	11.0	16.2	
May 2	43	1.3	7.8	540	4.4	2.5	885	39	54	10.7	0.3	2.9	11.4	13.4	105 (May 1–3)
4							795	29	49	11.1	0.3	2.9	12.0	15.0	100 (May 4–5)
6							1,231	71	100	10.5	0.1	3.2	12.7	14.8	
8	30	1.1	5.8	374	4.5	2.7	1,417	110	116	4.8	0.1	2.0	11.6	—	
10							1,075	56	64	4.8	0.4	2.8	8.3	14.0	
12							685	14	33	11.4	—	1.8	9.5	15.3	100 (May 12–16)
14							890	6	31	12.9	3.1	2.2	11.0	13.7	
16	34	1.3	4.7	389	401	2.6	1,120	11	46	19.6	4.3	4.4	13.2	15.5	
18							2,722	113	119	20.2	2.9	2.7	15.8	18.0	
20							2,365	121	123	9.5	1.2	3.1	7.4	16.4	
22	24	1.7	4.5	368	4.7	3.2									
June 6	19	1.2	5.6	361	5.1	3.2									

A.M., (Table 9), a 17-year-old male, was admitted to the hospital in November 1949, complaining of massive generalized edema. He had been in good health until May of the same year, at which time he had had an upper respiratory infection which had led to the appearance of swelling of the face and ankles. The diagnosis of nephrosis was made, and the patient was admitted to Mount Sinai Hospital, New York City. The blood pressure was found to be elevated at this time, a finding which was not evident subsequently. In August, the patient suffered from hay fever with shortness of breath. Transfusions of albumin were given at this time. Anorexia became troublesome, and a low-grade fever appeared. These complaints persisted, associated with varying degrees of edema. Table 9 shows the results of two courses of ACTH

Course: Massive diuresis followed the course of treatment, on both admissions.

The serum urea nitrogen concentration showed only slight changes from the control value of 16.4 mg. per 100 ml. The blood cholesterol level declined substantially during and after treatment but failed to drop below 800 mg. per 100 ml. The urinary protein increased during administration of ACTH and decreased below control levels after discontinuing treatment. The value at the time of the second admission was significantly below the initial control value. The level of serum proteins rose slowly, but only to a modest extent. The urinary steroids, although determinations were not performed continuously, indicated an active response from the adrenal cortex.

Classification—partial remission. The follow-up history indicated that the patient underwent a relapse, at which time he was again treated with ACTH.

B.I., (Table 10), a 37-year-old Japanese male theological student, was admitted to the hospital on November 11, 1951, complaining of ascites and generalized edema of nine weeks' duration, for which he had received treatment at another hospital. Past history was noncontributory. Physical examination confirmed the presence of massive edema and ascites. The blood pressure was found to be 120/72. Urine analysis revealed hyaline and granular casts, insignificant numbers of cells, and a high concentration of protein. The blood counts were within normal limits; the erythrocyte sedimentation rate was 50 mm. in one hour. On admission the patient's weight was 62.5 Kg. which was estimated to be 11 Kg. in excess of his normal weight. The blood urea nitrogen value was 17.0 mg., serum creatinine 1.0 mg., cholesterol 520 mg., cholesterol esters 310 mg., total protein 3.3 Gm., and albumin 0.6 Gm. per 100 ml. Urinary protein loss varied from 4 to 5 grams per twenty-four hours. The patient was given a salt intake of 700 grams daily. Treatment consisted of adrenocorticotropin, 25 mg. in one liter of 5 per cent glucose solution, daily, by the intravenous route. Within ten days the body weight had decreased by diuresis to 43 Kg. The patient was discharged on December 12, at which time the proteinuria had diminished to a trace in twenty-four hours; the serum cholesterol value was 282 mg., total protein 6.8 Gm., and albumin 3.2 Gm. per 100 ml. By January 3, 1952, evidences of relapse were noted in the urinary protein excretion, which had risen suddenly to 10 grams in twenty-four hours, and in the serum cholesterol level of 340 mg. per 100 ml. The serum total protein concentration had decreased to 4.1 Gm. and that of albumin to 1.3 Gm. per 100 ml. The patient was rehospitalized on January 28, and treated as on the the previous admission. The second experimental period was characterized by steady improvement, and after eight days of treatment with corticotropin intravenously and approximately one month of 80 mg. daily intramuscularly, the dose was gradually diminished and then discontinued on May 1. The patient was discharged from the hospital on June 30, without evidences of further relapse, and was sent back to Japan in August.

TABLE 9. A.M.

Date	Serum						Urine							N intake (Gm./ 24 hrs.)	Body wt. (Kg.)	ACTH (I.U. per day)
	Urea N (mg./ 100 ml.)	Creat. (mg./ 100 ml.)	P (mg./ 100 ml.)	Chol. (mg./ 100 ml.)	Tot. prot. (Gm./ 100 ml.)	Alb. (Gm./ 100 ml.)	Vol. (ml./ 24 hrs.)	Na (mEq./ 24 hrs.)	Cl (mEq./ 24 hrs.)	17-KS. (mg./ 24 hrs.)	Cort. S. (mg./ 24 hrs.)	Prot. (Gm./ 24 hrs.)	N (Gm./ 24 hrs.)			
1949																
Nov. 30	16.4	1.1		1,423	3.5	0.7	660	33	45	14.1	1.3	6.3	11.3		80.5	
Dec. 2							590	9	25	7.8	2.3	4.8	11.8	14.3	80.0	
4							960	8	33	19.3	3.9	6.2	17.6	14.6	80.2	100 (Dec. 2–7)
6	17.0	1.6	4.1	1,268	3.4	0.7	1,130	12	64	—	—	5.0	17.2	20.3	80.1	
8							4,070	494	405	—	—	0.9	15.1	21.5	79.8	
10							6,560	945	758	—	—	0.6	11.0	22.9	71.6	
12	13.0	1.3	3.5	1,174	4.5	1.9	2,660	216	258	—	—	1.2	15.9	20.5	62.9	25 (Dec. 11–17)
14							1,310	13	62	—	—	2.4	19.1	26.4	62.0	
16							1,100	7	49	—	—	2.2	20.6	30.7	63.4	
18							1,285	16	56	—	—	2.8	19.9	39.9	65.4	
20	18.0	1.2	4.5	813	4.9	2.1	1,450	48	64	10.0	0.1	3.7	19.8	27.8	66.8	

TABLE 10. B.I.

Date	Serum						Urine							Body wt. (Kg.)	ACTH (I.U. per day)
	Urea N (mg./ 100 ml.)	Creat. (mg./ 100 ml.)	P (mg./ 100 ml.)	Chol. (mg./ 100 ml.)	Tot. prot. (Gm./ 100 ml.)	Alb. (Gm./ 100 ml.)	Vol. (ml./ 24 hrs.)	Na (mEq./ 24 hrs.)	K (mEq./ 24 hrs.)	Cl (mEq./ 24 hrs.)	17-KS (mg./ 24 hrs.)	Cort. S.* (mg./ 24 hrs.)	Prot. (Gm./ 24 hrs.)		
1951															
Nov. 7	17	1.0	2.5	520	3.3	0.6	750	57	14	35	4.8	3.8	4.5	62.5	
9							995	83	15	53	6.0	4.6	3.6	61.5	
11							1,510	56	36	52	11.2	9.3	2.2	59.6	25 I.V. (Nov. 11–16)
13							2,365	47	53	65	19.6	22.0	2.5	59.7	
15	20	0.6	3.0	590	3.9	0.8	3,315	192		256	16.9	9.2	2.6	58.6	
17							4,130	314	24	392	5.4	6.2	0.9	55.0	
20	16	0.6	3.6	413	4.7	1.4	2,510	103		170	8.5	7.0	1.2	44.8	
22							1,165	32	60	34	5.4	3.1	0.8	42.8	
24							1,340	13	63	21	9.4	4.6	1.0	43.1	
26							1,330	25	44	19	6.0	2.3	0.8	43.2	
28	21	0.9	3.8	370	5.4	2.0	1,135	22	53	27	10.5	1.0	1.1	43.7	
30							930	21	38	20	5.6	1.4	0.9	43.6	
Dec. 3	13	0.9	4.1	328	6.4	2.2									
12	17	1.1	4.8	282	6.8	3.2									

* Glucuronidase method.

A.D., a 22-year-old male, had been in robust health until 1945, when the discovery of protein in the urine led to his discharge from the Navy. The patient noted no symptoms, however, until October 1948, at which time massive generalized edema, impairment of vision, elevation of blood pressure and convulsive seizures developed. He was hospitalized and given blood transfusions and intravenous glucose solution. In January 1949, he was admitted to Passavant Memorial Hospital for study and treatment. On January 24 and 25, a dose of 25 mg. of ACTH was administered intramuscularly every six hours for a total of six injections. The patient gained several pounds during this interval; however, the subsequent two weeks were associated with a diuresis of 5.5 Kg. The blood cholesterol level dropped from 650 mg. to 358 mg. per 100 ml. and the patient experienced an impression of definite clinical improvement. Readmitted to the hospital in June, 1949 he was treated with 100 mg. daily in divided doses for nine days, following which course a prolonged diuresis of 13 Kg. took place, accompanied by a decrease in the serum nitrogen level and a compensation of acidosis. Table 11 describes a later (1950) experimental period which was undertaken after a general relapse had occurred.

Course: Manifest edema was absent at the time of this 1950 admission. Administration of ACTH resulted in a weight gain of approximately 3 Kg., which was lost during the diuresis which followed the course.

Although this patient entered the hospital in a state of profound uremia with acidosis, the results of three courses of ACTH in a period of three months were good, and the serum urea, creatinine and phosphorus concentrations were reduced to about one half of the initial values. As on previous admissions, with similar treatment, the erythrocyte count and hemoglobin level improved markedly, and the acidosis subsided. The serum cholesterol level was not strikingly abnormal at this time, and changes were negligible. The serum proteins were slow to change but had increased substantially by the end of March. The urinary protein tended to increase during the diuresis following the course, paralleling the negative nitrogen balance. The corticoid response was sluggish, in general.

Classification—partial remission in chronic nephritis. The follow-up history indicated that the patient had severe epistaxis several months after discharge. He was admitted to a neighborhood hospital as an emergency, where he died several days later.

A.G., a 42-year-old married woman was admitted to the hospital on October 2, 1950, complaining of marked swelling of the lower extremities and puffing of the face, of approximately seven months' duration. The past history was uneventful except for scarlet fever and diphtheria in childhood, and the patient believed herself to be in excellent health until March 1950, when she found that her ankles were somewhat swollen. The swelling disappeared but was again noted in April. A diagnosis of nephrosis was made at this time and the patient was advised to take a diet high in protein and low in salt. Physical examination on admission showed marked edema of the lower extremities extending above the hips. There were no other significant findings. The blood pressure was 155/96. The erythrocyte count was 3,990,000, the leucocyte count 4,200, and the hemoglobin level 11.7 Gm. The urine contained protein, casts, and a few red and white cells. The patient was given two successive courses of ACTH and was discharged November 4, 1950. The data presented in Table 12 were derived from a subsequent period of observation and treatment in 1951.

Course: The intramuscular course presented in Table 12 shows negligible results in terms of water and salt excretion, and contrasts with the three-day intravenous course which involved a gain in body weight of approximately 5.5 Kg., with a subsequent diuresis of 12 Kg.

TABLE 11. A.D.

Date	*Serum*						*Urine*							N intake (Gm./ 24 hrs.)	Body wt. (Kg.)	ACTH (I.U. per day)
	Urea N (mg./ 100 ml.)	Creat. (mg./ 100 ml.)	P (mg./ 100 ml.)	Chol. (mg./ 100 ml.)	Tot. prot. (Gm./ 100 ml.)	Alb. (Gm./ 100 ml.)	Vol. (ml./ 24 hrs.)	Na (mEq./ 24 hrs.)	Cl (mEq./ 24 hrs.)	17-KS. (mg./ 24 hrs.)	Cort. S. (mg./ 24 hrs.)	Prot. (Gm./ 24 hrs.)	N (Gm./ 24 hrs.)			
1950																
Feb. 7	89	15.4	12.2	302	4.0	1.9	1,680	40	34		0.3	4.5	10.3	8.0	71.4	
9							2,000	39	48			5.9	12.0	8.8	70.9	
11							1,282	25	30			4.5	7.9	11.6	70.9	50 (Feb. 11–)
13							1,100	8	21			2.4	7.3	12.8	72.0	75 (Feb. 12–18)
15	118	14.0	8.8	405	4.4	2.2	1,425	14	26				10.2	12.7	72.7	
17							1,407	17	24		1.5	4.4	10.7	11.1	73.9	
19							1,355	14	36		1.3	3.7	10.1	9.2	73.9	
21	122	8.3	8.4	305	4.6	2.5	1,694	21	54		1.3	6.1	11.8	11.9	73.6	
23							2,275	49	73		0.4	8.2	10.8	13.1	72.7	
25							2,023	69	84		0.4	5.9	9.9	13.6	71.8	
27							1,975	81	96		0.4	4.7	8.9	13.3	71.3	
Mar. 1	70	9.6	6.1	316	4.6	2.4	1,970	75	93		0.2	4.2	8.4	12.1	71.3	
3							1,884	72	82		0.1	4.5	8.4	11.5	69.8	
5							1,845	88	88		0.1	5.2	10.0	13.2	68.6	
7	50	9.5	6.5	320	4.2	2.6	1,666	80	86		0.2	6.0	7.5	12.9	67.7	
14	47	8.7	5.1	368	4.1	2.7										40 (Mar. 14–28)
21	57	8.5	5.9	405	5.2	3.3										
28	44	8.1	7.3	345	5.5	3.3										

TABLE 12. A.G.

Date	*Serum*						*Urine*							N intake (Gm./ 24 hrs.)	Body wt. (Kg.)	ACTH
	Urea N (mg./ 100 ml.)	Creat. (mg./ 100 ml.)	P (mg./ 100 ml.)	Chol. (mg./ 100 ml.)	Tot. prot. (Gm./ 100 ml.)	Alb. (Gm./ 100 ml.)	Vol. (ml./ 24 hrs.)	Na (mEq./ 24 hrs.)	Cl (mEq./ 24 hrs.)	17-KS. (mg./ 24 hrs.)	Cort.* S. (mg./ 24 hrs.)	Prot. (Gm./ 24 hrs.)	N (Gm./ 24 hrs.)			
1951																
Feb. 12	18	1.1	4.7	550	3.3	0.9	2,360	53	80	11.1	5.1	8.5	8.5	11.9	71.9	
20	20	1.5	5.3	510	3.8	0.9	2,105	3	12	19.4	7.3	16.7	6.2	12.1	69.5	160 I.M. (Feb. 3–9)
22							2,085	4	13	13.0	13.1	10.5	10.5	12.1	69.1	
24							2,075	5	14	26.9	6.6	10.4	7.7	13.7	69.1	
26	27	1.3	5.8	630	4.8	1.0	1,730	3	11	24.9	8.7	14.9	8.5	12.6	69.1	
28							2,250	3	13	29.6	7.9	12.1	14.1	13.3	70.0	
Mar. 2							2,405	17	36	20.7	10.5	6.0	11.2	14.2	70.0	
4							1,965	27	36	13.7	4.8	9.9	11.7	13.7	70.0	
6	18	1.2	5.2	480	3.6	1.2	1,680	23	27	8.4	4.4	16.4	9.5	13.2	69.1	
8							1,530	16	28	11.9	6.8	11.0	8.4	12.5	69.1	50 I.V. (Mar. 9–11)
10							1,905	2	13	32.5	13.6	14.8	10.2	11.0	73.6	
12	38	2.4	6.2	480	3.2	0.9	4,450	23	56	39.6	20.2	12.8	13.5	13.5	74.5	
14							2,575	109	175	24.3	14.3	6.5	12.5	12.8	69.1	
19	20	1.5	6.2	430	3.8	1.0	2,250	58	91	10.0	10.0	15.8	10.0	12.5	65.0	
27	19	1.7	6.4	565	3.5	1.2	2,740	76	131	—	—	19.7	10.4	11.0	62.7	

* Glucuronidase method.

Changes in the serum cholesterol concentration were present but not striking. The level of serum proteins showed no increase. The urinary protein loss was large at all times, and tended to be greater over the reported period. The urinary 17-ketosteroids and corticoids were low during all courses employing the intramuscular route but were somewhat higher with the intravenous route. The high level was considerably prolonged after the brief intravenous course.

Classification—clinically unimproved by treatment.

J.McL., (Table 13), was a 37-year-old male, who had been in good health until August 1947, at which time he noticed swelling of the ankles in the evening. He was treated with Salyrgan and ammonium chloride until he was admitted to the Mayo Clinic in April 1948. A diagnosis of nephrotic syndrome was made at this time. In November, ascites developed and paracentesis was performed on several occasions. When admitted to Passavant Memorial Hospital in August 1949, edema and ascites were marked. The value for serum urea nitrogen was 29 mg. cholesterol 635 mg., total serum proteins 4.9 Gm. and albumin 1.2 Gm. per 100 ml.

He was started on a salt intake of 700 mg. daily and treated with ACTH intramuscularly in four courses, one of which (November 1949) is indicated in Table 13. With the exception of the period following the third course, during which there was a moderate increase in the excretion of sodium and chloride and sufficient output of water to account for a total weight loss of 5.5 Kg., this patient showed minimal modifications in the chemical elements of the blood and urine. He appeared to be unresponsive to the medication.

R.B., (Table 14), a 48-year-old married man, was admitted to Passavant Memorial Hospital on March 19, 1950, complaining of proteinuria of two years' duration, with recent fatigue and headaches. The childhood history was uneventful; he had undergone appendectomy and cholecystectomy in 1926. The patient stated that he had had "ulcerative colitis" for the preceding thirty years, with blood and mucus in the stools. This condition was said to become exaggerated in times of nervous tension. Upon admission to the hospital, the patient was found to be in good nutritional condition, and there were no abnormal physical findings. The blood pressure was 118/80. The blood counts were not diminished; the urine showed protein but no casts or blood cells. Radiologic studies of the colon revealed no evidence of ulceration. An intravenous pyelogram indicated prompt renal excretion of the dye and generally normal contours of the urinary tract. The patient was given several intramuscular courses of ACTH, one of which is presented in Table 14.

Course: No edema was present and changes in body weight during treatment were slight.

The control serum urea nitrogen level of 23 mg. per 100 ml. rose somewhat during treatment and declined to 15 mg. per 100 ml. after conclusion of treatment. The serum cholesterol level declined, and that of serum proteins rose substantially after each course. The urinary protein excretion increased during administration of ACTH and returned to control values after omission of treatment. The urinary steroid excretion was low in general but increased somewhat with successive courses.

Classification—partial remission. Follow-up studies, interspersed with repeated courses of treatment indicated that serum proteins and cholesterol tended to return to original control levels after from six to eight weeks without medication. In the eleven months succeeding his first hospital admission, the level of protein excretion in the urine increased by 1 to 2 grams per twenty-four hours.

TABLE 13. J.McL.

Date	*Serum*						*Urine*							N intake (Gm./ 24 hrs.)	Body wt. (Kg.)	DCA (mg.)	ACTH (I.U. per day)
	Urea N (mg./ 100 ml.)	Creat. (mg./ 100 ml.)	P (mg./ 100 ml.)	Chol. (mg./ 100 ml.)	Tot. prot. (Gm./ 100 ml.)	Alb. (Gm./ 100 ml.)	Vol. (ml./ 24 hrs.)	Na (mEq./ 24 hrs.)	Cl (mEq./ 24 hrs.)	17-KS (mg./ 24 hrs.)	Cort. S. (mg./ 24 hrs.)	Prot. (Gm./ 24 hrs.)	N (Gm./ 24 hrs.)				
1949																	
Nov. 3							440	11	6		0.5	4.6	7.0	7.1	104.5		
5							370	1	1		0.6	5.5	6.5	9.3	94.5†		
7	9	1.5	5.0	446	4.3	1.2	500	1	7		0.7	6.6	7.5	11.8	94.1	10	
9							745	1	8		0.7	9.3	9.8	12.8	95.4		
11							600	4	7		1.3	10.3	9.1	10.7	96.4	10	
13							370	2	5		2.6*	7.5	6.7	13.6	98.1	10	100 (Nov. 11–14)
15	18	2.0	4.9	536	4.3	1.3	620	3	7		4.9	5.6	11.0		99.3		120 (Nov. 15–16)
17							590	3	7		5.3	11.1	9.0		100.2		150 (Nov. 17–19)
19							670	4	16		4.9	8.8	4.3		101.8		
21							690	20	40		2.1	5.5	12.9		101.6		

* Glucuronidase method for this and subsequent values.
† Paracentesis.

TABLE 14. R.B.

Date	*Serum*						*Urine*							N intake (Gm./ 24 hrs.)	ACTH (I.U. per day)
	Urea Date (mg./ 100 ml.)	Creat. N (mg./ 100 ml.)	P (mg./ 100 ml.)	Chol. (mg./ 100 ml.)	Tot. prot. (Gm./ 100 ml.)	Alb. (Gm./ 100 ml.)	Vol. (ml./ 24 hrs.)	Na (mEq./ 24 hrs.)	Cl (mEq./ 24 hrs.)	17-KS (mg./ 24 hrs.)	Cort. S. (mg./ 24 hrs.)	Prot. (Gm./ 24 hrs.)	N (Gm./ 24 hrs.)		
1950															
Mar. 27	23	2.4	3.8	610	5.2	2.2	2,340	144	149	11.2	0.1	2.4	13.2	13.8	
29							1,975	49	50	9.0	0.2	2.7	14.4	13.9	35 (Mar. 29–)
31							2,050	57	59	9.5	0.4	2.9	14.2	19.6	70 (Mar. 30–31)
Apr. 2							2,050	59	62	15.2	0.3	3.1	15.6	16.7	105 (Apr. 1–2)
4	28	2.3	4.3	560	5.3	2.3	1,795	66	50	15.2	0.3	3.0	15.4	18.5	100 (Apr. 3–4)
7							2,272	130	110	17.9	0.1	4.2	15.6	18.4	
9							1,720	42	40	9.6	0.1	3.9	13.1	15.5	
11	15	1.8	4.1	355	5.2	3.0	1,425	17	41	8.3	0.4	4.4	12.4	16.1	
13							1,675	17	56	8.5	0.3	3.6	12.4	16.4	
15							1,610	29	66	9.1	0.1	2.9	11.3	17.5	

L.B., (Table 15), a 58-year-old woman, entered Passavant Memorial Hospital in August 1950, complaining of massive swelling of the feet and legs of approximately one year's duration, and known proteinuria. With the exception of the edema, physical examination was noncontributory. The blood pressure was 100/68. Routine blood counts were within normal limits. Intravenous pyelography revealed no abnormality of the urinary tract, and blood cells were absent from the urine. Two courses of ACTH were given.

Course: Changes in over-all body weight were insignificant during and after the two courses. However, the marked edema of the lower extremities disappeared, and there has been no recurrence.

The serum cholesterol concentration remained essentially constant throughout the entire period of observation. The values for serum proteins underwent only slight modifications. The urine protein loss diminished somewhat, and the decrease persisted during the nine-month follow-up period. Corticosteroid excretion was considered average.

Classification—clinically inconclusive. Follow-up studies showed that proteinuria was no longer present. The serum cholesterol level was 302 mg. per 100 ml.; the levels of serum proteins were within normal limits and the patient had no complaints.

SUMMARY OF CLINICAL RESULTS

Sodium, chloride and water. In those patients who did not have manifest edema (R.B., L.T. and G.H.—Tables 14, 4 and 8) before treatment there was little tendency for edema to develop while they were receiving ACTH. The conditions under which the studies were conducted were not closely enough controlled with respect to salt intake to permit of conclusions in terms of sodium balance. All subjects were given a 700-mg. sodium diet, but failure to consume the full dietary allowance often decreased the daily consumption, and the over-all increase which was requested during intervals of increased appetite resulted in an unavoidable increase in sodium intake. In any event, R.B. and G.H. were grossly in equilibrium or even in negative balance during the control period, and the decrease in sodium excretion was slight in both cases in comparison with the rest of the group. Alterations in fluid intake and output were proportionately slight. In many (Tables 1, 2, 3, 5, 10 and 13) the control period was characterized by minimal excretion of water and salt and in these instances no further retention could be demonstrated.

Diuresis was established in all edematous cases, with 3 exceptions (M.L.M., Table 6; J.McL., Table 13; and L.B. Table 15) during or after a course of varying duration. In 1 instance (D.D., Table 5) body-weight figures were omitted because paracentesis had been performed prior to the experimental period reviewed in Table 5; the diuresis can best be evaluated by the excretion figures. In 2 patients (Tables 3 and 10) diuresis with complete remission took place during administration of ACTH. In 8 patients (Tables 1, 2, 5, 6, 7, 9, 11 and 12) profound diuresis followed the course of treatment; in 7 of these there was a substantial decrease in the level of serum cholesterol and a varying improvement in proteins.

TABLE 15. L.B.

Date	*Serum*						*Urine*							Body wt. (Kg.)	ACTH (I.U. per day)
	Urea N (mg./ 100 ml.)	Creat. (mg./ 100 ml.)	P mg./ 100 ml.)	Chol. (mg./ 100 ml.)	Tot. prot. (Gm./ 100 ml.)	Alb. (Gm./ 100 ml.)	Vol. (ml./ 24 hrs.)	Na (mEq./ 24 hrs.)	K (mEq./ 24 hrs.)	Cl (mEq./ 24 hrs.)	17-KS (mg./ 24 hrs.)	Cort. S.* (mg./ 24 hrs.)	Prot. (Gm./ 24 hrs.)		
1950															
Aug. 22	18	0.9	4.8	405	6.3	2.3	1,268	30	37	33	2.9	2.1	3.0	72.0	
24							1,437	9	63	24	12.0	5.4	5.7	70.7	100 (Aug. 24–
26							1,575	5	55	21	17.6	6.7	4.7	71.5	Sept. 1)
28	20	0.9	5.5		6.2	1.9	1,600	3	50	17	7.8	3.5	5.8	71.6	
30							1,815	5	56	15	13.1	15.2	4.1	71.8	
Sept. 1							1,497	13	36	14	12.9	5.4	1.5	71.3	
3							1,530	31	52	16	7.0	2.7	2.2	71.8	
5	15		4.1	440	5.6	2.1	1,697	10	75	14		3.9		70.9	
7							2,085	1	64	15	22.7	6.3		71.1	100 (Sept. 6–15)
9							1,927	3	42	20	20.1	7.9		71.2	
11	16	1.2	4.5	450	6.0	2.3	1,890	6	62	16	28.6	17.9		71.3	
13							1,955	8	88	18	18.4	6.9	3.3	71.8	
15	16	1.0	4.3		5.6	1.9	1,932	33	51	23	10.3	2.5	2.4	70.9	
Nov. 14	15	1.2	4.3	412	6.5	2.3	1,380			124			3.0		
1951															
Jan. 21							1,170	111		114			0.7		
Feb. 18							760	81		96			1.7		
Apr. 15							1,180			48			0.8		

* Glucuronidase method.

Serum sodium, potassium, chloride and phosphorus. No consistent trend was noted in the values for these elements.

Serum cholesterol. In some instances there was a brief primary rise in the serum cholesterol level after ACTH was started, which gave way to a marked decrease. Frequently the decrease was greatest during a period of several weeks following withdrawal of ACTH. It was also greatest when the control level was exceedingly high (Tables 1, 2, 3 and 5).

Serum urea nitrogen. In 1 case (Table 2) the serum urea nitrogen level decreased during the period of administration and continued its downward course after ACTH was discontinued. In 8 cases there was no significant change. In the other 6 cases there was a definite increase, which subsequently gave way to concentrations comparable to, or below those of the control level. The behavior of the serum urea nitrogen concentration could not be correlated with that of creatinine, nor did it appear to be associated with changes in water and salt excretion or with the filtration rate as calculated on the basis of endogenous creatinine.

Proteinuria. In many instances proteinuria increased when ACTH was administered, and decreased when it was discontinued.

Nitrogen balance. Changes in nitrogen balance observed in this group were not striking. In the majority of patients the urinary nitrogen (allowing a small constant increment for fecal nitrogen) indicated a negative balance, which was apt to appear around the fourth day and to continue for several days. The total loss, however, was small and rarely exceeded 5 or 6 grams. The long period of nitrogen retention which was apt to follow corresponded to the interval during which the concentration of serum proteins increased. (A.M. furnished a good example of such a period, although economy of space made it unfeasible to include the data with Table 9.) It is to be noted, however, that the restoration of serum proteins was by no means an invariable consequence of treatment, and in several subjects a profound diuresis with a marked decline in the level of serum cholesterol failed to lead to a protein anabolic effect as reflected by an increase in the concentration of serum proteins.

Urinary corticosteroids and 17-ketosteroids. For the clearer evaluation of the control corticoid levels as an index to the status of the adrenal cortex in the untreated nephrotic state, values found in our series have been plotted in Figure 1 with reference to age group, against reported values obtained by the same method in normal individuals (8). Inspection shows that the values in the nephrosis series fall within the limits of normal. Results obtained by the same method after hydrolysis with beta-glucuronidase showed an approximate tenfold increase. Although normal values obtained by the same method for comparable ages are not available, it is improb-

able that the nephrosis patients would show an important trend beyond the normal.

Values for 17-ketosteroids were low in several of our patients during the control periods. Since other members of the group showed normal levels, the fact is of dubious significance.

The degree of activity elicited from the adrenal cortex by the administration of ACTH is suggested by a corticoid output which varied from an

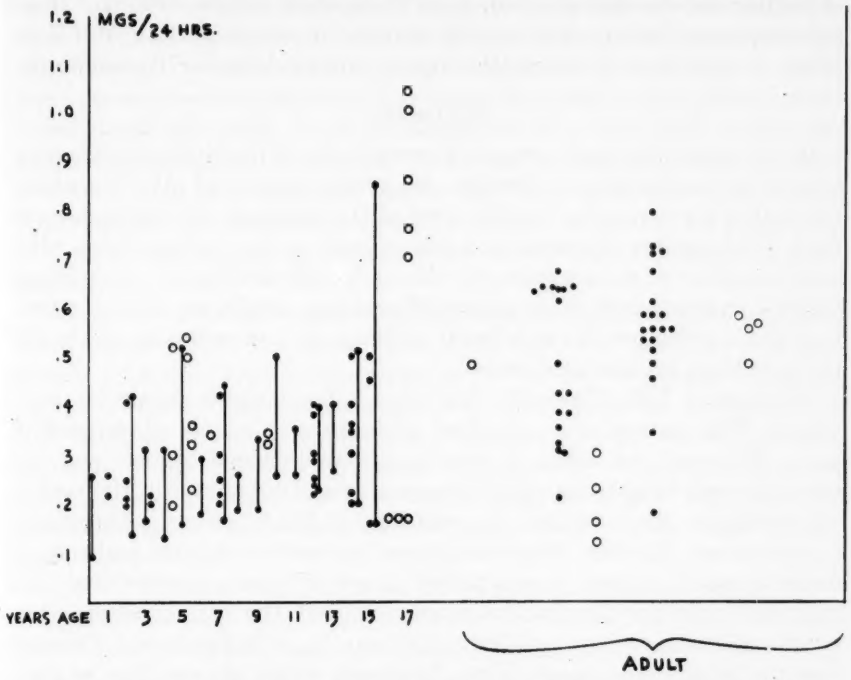

FIG. 1. Values for formaldehydogenic corticoids in relation to age of patients. Dots indicate values for normal individuals (8); open circles indicate control values for patients with nephrosis, prior to treatment.

increase of slightly more than twofold (M.L.M., Table 6, first course of treatment) to one of fortyfold or more (L.T., Table 4). It must be pointed out, however, that the test for formaldehydogenic corticosteroids picks up a number of substances which are physiologically inactive, and the values obtained are therefore augmented by steroids which bear an undetermined relationship to those of known physiologic significance. This is particularly true when enzyme hydrolysis is employed. Subsequent work in this laboratory has shown that paper chromatograms run on duplicate

aliquots, one of which was pretreated with beta-glucuronidase, resulted in a liberal increase in the enzyme-treated portion, not of compounds E or F, but of a compound more polar than F. It was concluded that enzyme hydrolysis serves to multiply an inactive increment and to distort the values obtained by the test.

In general, the rise in urinary 17-ketosteroids appeared somewhat later than did the rise in corticoids, and was apt to persist longer after the course of medication was discontinued. Notwithstanding certain strikingly sluggish responses, the group as a whole responded competently to ACTH in terms of excretion of formaldehydogenic corticoids and 17-ketosteroids.

DISCUSSION

In the group of patients reported here a chain of reactions took place in several metabolic fields following the administration of ACTH, which resulted in the reversal or modification of the nephrotic syndrome in each field. The sequence of events is shown to begin at the renal tubules, with an increase in the reabsorption of sodium, chloride and water—a condition which is characteristic of the untreated nephrotic syndrome. Thus the first result of stimulation of the adrenal cortex is an augmentation of the already existing tubular dysfunction.

The case of L.B. (Table 15) illustrates a point which should be mentioned. This patient was admitted with massive edema of dependent parts. Although she failed to experience a well-defined diuresis and decrease in body weight, the edema disappeared and did not recur. Her course was evidently characterized by migration of fluid into the intracellular compartment. Routine determinations of potassium in urine and serum, made in many patients over a period of several years, suggest that this migration involves the potassium ion and that the hypokaliemia often cited is due less to increased excretion than to re-inclusion of potassium into the intracellular space. Thus the events which are manifest as they occur in connection with the renal tubules, may well be operative throughout the tissue spaces.

Chronologically, following these modifications of ionic exchanges in the renal tubules and presumably over the membranes enclosing the intracellular fluid space, changes in lipid metabolism occur, which are reflected in the decline of the serum cholesterol level. In several instances (R.F., Table 2, for example) the cholesterol level rose before it began to fall. As we have reported elsewhere (9), the changes in cholesterol concentration are less marked than those involving fatty acids and triglycerides.

The impression gained by reviewing this series of cases as influenced by adrenocorticotropin is that the hormone is capable of intensifying specifi-

cally all the characteristics of the nephrotic syndrome. This effect leads to a more or less comprehensive remission of variable duration. In the field of lipid metabolism, at least with respect to serum cholesterol concentration, the changes evoked are exaggerations of known actions of ACTH, as are those affecting electrolytes and water. The intensifying of proteinuria is reminiscent of the studies of Addis and his group (10). The physiologic correction of the concentration of serum proteins, however, does not appear to be a direct effect of the cortical hormones (11) and we were unable to show by electrophoretic studies that a particular component was specifically affected by cortical stimulation. The records showed, rather, a general recovery from the depleted and distorted serum protein pattern characteristic of the disease, in those cases which responded well in other respects. The mode and the timing of the effects upon the blood proteins, indicating that the corticoids themselves do not directly produce the improvement, are consistent with the opinion currently held that the cortical hormones are both antianabolic and catabolic (12), but in no sense anabolic.

Correlation between evidences of glandular activity and clinical results is complicated by a number of factors, the first of which is the fact previously mentioned that values obtained by the procedure for the determination of formaldehydogenic steroids include those for substances of undetermined metabolic significance. Moreover, because of the collective character of the test, no information is gained concerning the qualitative nature of cortical response. We obtain a gross total from a gland which possesses multiple active principles, without description or measurement of the components.

Another probable factor involves the condition of the end-organ.

Such factors may account for the variable relationship found between clinical course and corticosteroid values. For example, 2 patients in whom a short course of treatment brought about a complete remission (K.W.D., Table 3; and L.T., Table 4) showed a liberal corticoid response, with values far higher than all others obtained without the use of glucuronidase. Conversely, J.McL., whose clinical course was unsuccessful, gave an exceedingly modest corticoid response to ACTH, 150 units daily, administered intramuscularly in divided doses at six-hour intervals. Between these two extremes, however, lie such records as that of T.K. (Table 1) in which high and sustained corticoid values coincided with relatively unsatisfactory clinical progress, and that of R.F. (Table 2) in which insignificant steroid response was associated with satisfactory clinical improvement. In the first of these 2 patients, the long duration of the disease prior to treatment may have affected the result, through changes in the end-organ.

Whatever the explanation for such apparent discrepancies, it must be concluded that the evaluation of adrenocortical activity supplied by the determination of urinary 17-ketosteroids and formaldehydogenic corticosteroids fails to show a characteristic dysfunction of the gland, either in the quiescent state or under stimulation by ACTH.

SUMMARY AND CONCLUSION

Records of 15 patients suffering from the nephrotic syndrome are presented, which show in detail the results upon blood and urine metabolites of treatment with adrenocorticotropic hormone. The data include determinations of urinary formaldehydogenic steroids and 17-ketosteroids, and furnish therefore a reflection of cortical activity in conjunction with the indicated metabolic changes associated with treatment.

The 2 patients who responded to a short course of treatment with complete clinical recovery showed values for corticosteroids which were far higher than those of the other patients constituting the experimental group. Conversely, the poorest clinical results were associated with low steroid excretion values. Between the two extremes, however, no consistent correlation could be found between corticosteroid values and clinical progress. Control values for corticosteroids, determined before treatment was started, were within normal limits.

Changes involving the levels of serum cholesterol, proteins and nonprotein products, and excreted salt, water and protein conformed to those described by ourselves and other observers.

It is concluded that the relationship between the pituitary-adrenal complex and the nephrotic syndrome implied by the substantial accumulation of favorable clinical reports on the administration of ACTH in that syndrome, is inadequately described by estimates of 17-ketosteroid and formaldehydogenic steroid excretion.

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CHANGES IN SERUM VITAMIN A LEVEL DURING THE HUMAN MENSTRUAL CYCLE*†

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THE purpose of the present investigation was to study the variations of serum vitamin A concentration in normal women in relation to the menstrual cycle.

The wide variety of reports in the literature indicates that the equilibrium which exists between serum levels and liver stores of vitamin A is influenced by hormones (1–8). For example, Reichstein's compound L (3,β-acetoxy-17-α-hydroxy-allopregnan-20-one) appears to alter the equilibrium between blood and liver "A," resulting in a decreased level of serum vitamin A and increased liver stores (1). Stimulation of the sympathetic nervous system with adrenaline increases the vitamin A content of the blood and mobilizes vitamin A from the liver (2). Combined injections of estradiol and testosterone have been reported to produce a threefold to fourfold increase in the concentration vitamin A in the blood serum of immature pullets (3). When growth hormone is given to vitamin A-deficient rats, a subacute deficiency of the vitamin is converted to an acute deficiency (4). Sexual differences have been reported both in storage and in serum levels of vitamin A in experimental animals (5, 6) and in human beings (6–8). That these are related to hormonal differences between male and female is suggested in a report indicating that the site of storage of vitamin A in rats is influenced by sex hormones (6). These findings suggest that the hormonal variations during the menstrual cycle may bring about a correlated sequence of changes in the level of serum vitamin A.

EXPERIMENTAL

Six young women ranging from 18 to 23 years of age, who had no history of menstrual disorder, and 2 women in the same age group who had cyclic abnormalities, were chosen as subjects. Blood specimens were collected

Received for publication April 15, 1953.

* Presented in part before the Division of Biological Chemistry, American Chemical Society, 121st National Meeting, April 2, 1952, Milwaukee, Wisconsln.

† These studies were supported by Endo Products, Inc., and by the National Vitamin Foundation.

every second day, and daily during the menstrual period, for one to six months. Blood was taken in the morning prior to the intake of food. No dietary restrictions were imposed. Serum vitamin A values were determined using the methods of Sobel and Snow (9) and of Bessey *et al.* (10).

Large doses of vitamin A were administered orally to 3 of the 6 normal subjects in order to determine whether the magnitude of change in serum levels during the menstrual cycle would be affected when "A" stores were increased. Subject I received 70,000 USP units of vitamin A (Vifort capsules) forty-nine days prior to the study, 70,000 USP units of another preparation of "A" (Unicaps) thirty-seven days prior to the study, and 70,000 USP units of "A" (oleum percomorphum) on the first day of the study, but none in subsequent cycles. Subject IV received 125,000 USP units of vitamin A (Vifort drops) at the beginning of the second cycle, but no supplement in cycle I. Subject V received 125,000 USP units of "A" (Unicaps) one hundred and twenty-six days prior to the study, 125,000 USP units of "A" (Vifort capsules) one hundred and twenty-three days prior to the study, and 125,000 USP units of "A" (oleum percomorphum) forty-six days prior to the study.

RESULTS AND DISCUSSION

The data indicate that there is a regular cyclic pattern of vitamin A concentrations in the blood serum of normal women. Serum vitamin A levels were low during menstruation, rose to a peak about the fourteenth day of the cycle, receded, and then rose again until a second peak was observed on or about the twenty-fifth day (Table 1, Fig. 1). The changes were

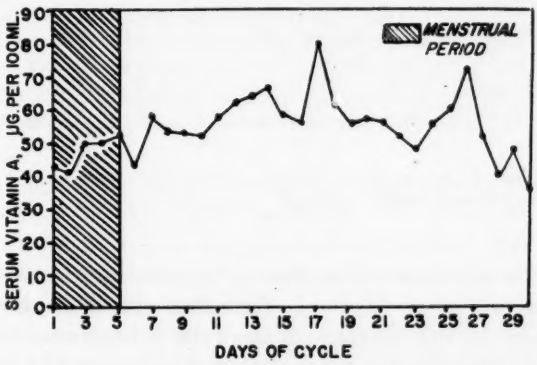

FIG. 1. *Fasting serum vitamin A levels during the menstrual cycle (mean values obtained from 14 normal cycles).* In plotting this graph, all cycles were equalized to thirty days, the calculated mean length of the observed cycles (see Table 1). Thus, the days on which peak "A "levels occur in Figure 1 do not coincide with the days on which the mean maximum levels occur in Table 1.

TABLE 1. CHANGES IN SERUM VITAMIN A LEVELS DURING 14 MENSTRUAL CYCLES IN 6 NORMAL SUBJECTS

Subject No. & age (yrs.)	Cycle	Length of cycle (days)	SERUM VITAMIN A							Vitamin A supplement
			Min. level		Peak I		Peak II		Max. increase	
			μg./100 ml.	Day of cycle	μg./100 ml.	Day of cycle	μg./100 ml.	Day of cycle	μg./100 ml.	
I* 22	a	34	17	3	86	12	52	27	69	70,000 USP units 49 days before study;† 70,000 USP units 37 days before study;‡ 70,000 USP units 3rd day of cycle§
	b	28	22	6	108	16	94	25	86	None
	c	32	12	3	72	16	51	28	60	None
	d	28	40	6	54	18	59	24	19	None
	e	29	43	3	84	17	80	24	41	None
	f¶	27	47	5	70	10	66	25	23	None
II 24	a	31	30	5	57	17	—	—	27	None
	b	31	42	1	78	13	72	27	36	None
III 23	a¶	35	40	3	62	17	56	29	22	None
	b	30	14	1	74	15	73	24	60	None
IV 19	a	23	31	5	46	14	—	—	15	None
	b	25	32	1	63	17	—	—	31	125,000 USP units 3rd day of cycle‖
V 23	a	29	16	1	86	6	84	17	70	125,000 USP units 126 days before study;‡ 125,000 USP units 123 days before study;† 125,000 USP units 46 days before study§
VI 18	a	40	35	1	103	18	84	25	68	None
Mean		*30*	*30*	*3*	*75*	*14*	*70*	*25*	*45*	

* Cycles a, b and c were consecutive; cycles d, e and f were also consecutive, beginning three months after cycle c.
† Vifort capsules.
‡ Unicaps.
§ Oleum percomorphum.
¶ Subject had upper respiratory infection during cycle.
‖ Vifort drops.

greater after the administration of massive doses of vitamin A than when no supplement was given (Table 1, Figs. 2 and 3). This phenomenon was probably caused by the interplay of the various hormones secreted during the menstrual cycle. In this connection it has been noted that one other substance for which cyclic patterns have been reported, namely citric acid (11–13), is sensitive to hormone injections (11, 14, 15). Patterns were obtained (11, 13) in which the trends of urinary excretion of citric acid during the menstrual cycle were similar to the present findings with serum

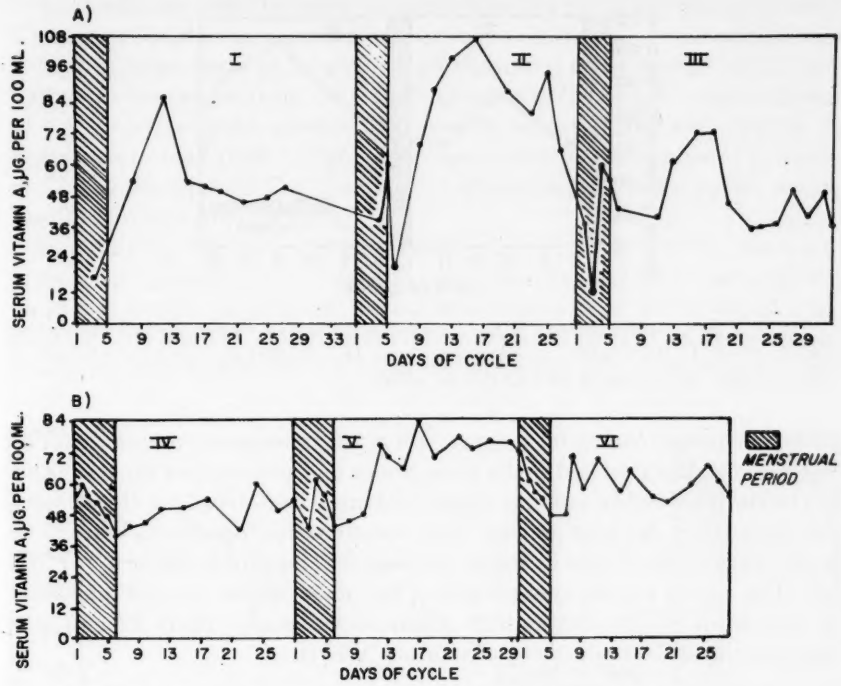

FIG. 2. *Fasting serum vitamin A levels during 6 menstrual cycles of normal subject.*

A. Three consecutive cycles—70,000 USP units of vitamin A (Vifort capsules) administered forty-nine days before study; 70,000 USP units (Unicaps) thirty-seven days before study; 70,000 USP units (oleum percomorphum) on first day of study.

B. Three consecutive cycles in same subject six months after cycle I. (See Subject I, Table 1.) No vitamin supplements administered.

levels of vitamin A (Fig. 1). Interestingly enough, the synthesis of citrate in the Krebs cycle is affected by hormones (15). However, a distinction must be drawn between the two metabolites, namely, that citric acid is synthesized within the body (15), whereas vitamin A is a trace nutrient derived solely from dietary sources.

Both abnormal cases failed to exhibit regular patterns of vitamin A levels. The first of these subjects reported a long history of premenstrual tension and dysmenorrhea. Menstruation took place at regular intervals of twenty-eight to thirty days. The serum vitamin A level fluctuated markedly from day to day, ranging between extremes of 20 and 125 micrograms per 100 ml. (Fig. 4). The second subject was oligomenorrheic, some-

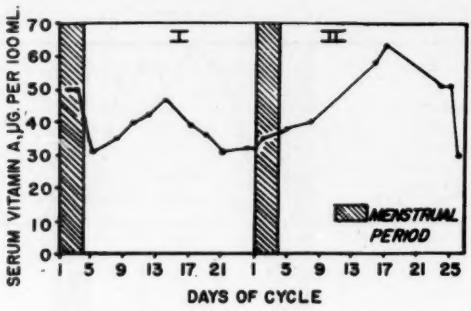

FIG. 3. *Fasting serum vitamin A levels during 2 consecutive menstrual cycles of normal subject.* Cycle I—no vitamin supplement. Cycle II—125,000 USP units of vitamin A (Vifort drops) administered on first day of cycle.

times not menstruating for as long as five to six consecutive months. The last menstrual period had taken place about one and one half months prior to the study. Serum vitamin A concentrations were followed in this subject for sixty-three days before the next menstruation occurred. Vitamin A levels were irregular, and ranged between 25 and 65 micrograms per 100 ml. (Fig. 5). On the thirty-sixth day of the observations, 125,000 USP units of vitamin A (Vifort drops) were administered orally. There was no subsequent change in the pattern of vitamin A levels.

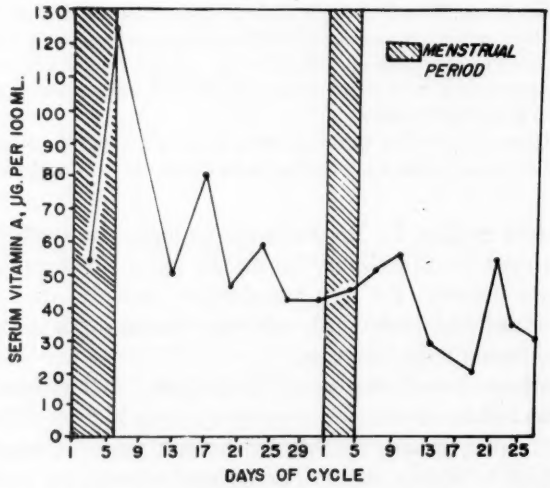

FIG. 4. *Fasting serum vitamin A levels during 2 consecutive menstrual cycles of subject with premenstrual tension and dysmenorrhea.*

Although one must be cautious in interpreting the observations on only 2 abnormal cases, the reported successful treatment of premenstrual tension with large doses of vitamin A administered daily during the second half of the menstrual cycle (16) may be related to the lack of cyclic patterns of "A", such as were observed in these 2 subjects. Massive vitamin A supplements may restore high blood levels and promote greater transfer of "A" to the reproductive tissues, thus alleviating the disturbances which result in premenstrual tension.

In obtaining data on cyclic patterns of serum vitamin A levels, there are pitfalls that must be understood and controlled. Factors other than regular hormonal secretions influence the concentration of "A" in the blood. One such factor is markedly elevated body temperature, such as occurs in febrile conditions, in which vitamin A levels are depressed (17). Upper respiratory

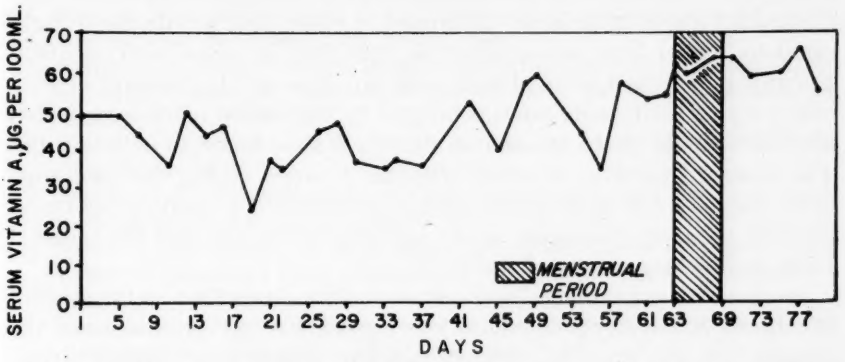

FIG. 5. *Fasting serum vitamin A levels in oligomenorrheic subject.* Last menstruation occurred about forty-five days prior to study—125,000 USP units of vitamin A (Vifort drops) administered on thirty-sixth day of study.

infection associated with fever was encountered in 2 subjects during the study. Cycles observed during the course of such an infection manifested a lesser degree of change in vitamin A levels than did other cycles in the same subjects (Table 1). Another factor which should be controlled is alcohol consumption. According to Clausen and his associates (18, 19), alcohol releases vitamin A from the liver stores into the blood, thus raising the serum level of "A." Preliminary studies in this laboratory indicate that serum vitamin A concentration increases after the consumption of an alcoholic beverage and that such changes are of sufficient magnitude to alter the cyclic pattern of the serum vitamin A level. However, these results are not conclusive.

The female reproductive system requires relatively large amounts of

vitamin A (17, 20–22). The endometrium, like all epithelial tissues, will not grow normally in the absence of "A" (17), and the vitamin is also required for the production of ova (20). The presence of "A" in the corpus luteum suggests that this gland requires vitamin A (22). Thus, the growth and maintenance of the endometrium, as well as the production of ova and corpora lutea, must depend on adequate supplies of vitamin A.

One may tentatively postulate that the net effect of the various hormones secreted during the menstrual cycle is such that in normal women maximal serum levels of vitamin A are reached when the requirement of the reproductive system is maximal, namely, in the pre-ovulatory phase for the production of reproductive cells and endometrium (17, 20), and in the post-ovulatory period for the maintenance of the endometrial layer; and that the levels are minimal when the requirement is at a minimum, during menstruation when the endometrium is being destroyed. This may account for a greater tendency of females to conserve liver vitamin A, indicated by higher liver stores in female rats than in males after identical amounts of "A" had been administered (6). That greater transfer of vitamin A across body membranes is favored by high blood levels is indicated in recent studies of the transfer of vitamin A from blood to milk (23–25). The observed patterns of serum vitamin A levels during the menstrual cycle may be a reflection of the varying amounts of "A" undergoing transfer from the liver storehouse to the reproductive tissues and the endometrium at different phases of the cycle.

Studies with experimental and farm animals indicate that, in the absence of vitamin A, fertility problems of varying degree result both in males and females (20, 21, 26). The severely deficient female may become sterile, with concomitant cessation of estrus (20). In less severe deficiency, mating occurs, but infertility or resorption of the young may result (21, 27, 28). In mild deficiency of vitamin A, breeding and conception take place, but abnormal *in utero* growth and development of the fetus are common occurrences (27, 28). An upset in the cyclic rise and fall of the serum vitamin A level may have some bearing on certain types of infertility in which ova are not produced or in which fertilized ova do not become securely implanted in the uterine wall. More elaborate investigations, specifically focussed on fertility problems, may elucidate the possible interrelation of cyclic variations of vitamin A and fertility.

SUMMARY

1. Cyclic changes in serum vitamin A levels were observed during 14 normal menstrual cycles. Minimal concentrations occurred during menstruation, and maximal concentrations about the fourteenth day of the cycle, and again about the twenty-fifth day.

2. Two subjects with abnormal menstrual cycles failed to manifest the changes observed in the normal women.

3. The observed cyclic fluctuations probably represent the transportation of increased amounts of vitamin A from the liver stores to the reproductive organs during the phase of endometrial growth.

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ABSORPTION OF CORTICOIDS AND RELATED STEROIDS FROM SUBCUTANEOUSLY IMPLANTED PELLETS IN THE GUINEA PIG

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INTRODUCTION

IN FORMER papers from this Department absorption of various steroid hormones from subcutaneously implanted pellets, calculated per square millimeter of the pellet (tablet), has been given for estradiol and its esters (1), for testosterone and its derivatives, and for progesterone (2).[1] Occasionally absorption per square millimeter has been given also for various corticoids (3, 4, 5). In the present paper new results, especially with substances hitherto not dealt with, are reported.

As to the *methods* used, and as to the *errors* to be considered, we refer to a former paper (2).

RESULTS

It is remarkable how little the results with progesterone in the present series (Table 1) differ from those in the former series (see Table 2 of (2)). The same is true for other substances mentioned in former papers; notable differences occur only when the number of available experiments is small (3, 4, 5).

The absorption rate of desoxycorticosterone acetate and its 17-hydroxy derivative (Reichstein's compound S) is not very far from that of progesterone. The absorption rate of either A or E is more than double the absorption rate of DOC or S. On the contrary, the absorption rate of F approaches that of DOC or S. Unfortunately we were not able to study the absorption rate of corticosterone (B) for comparison with that of F (17-hydroxycorticosterone).

Received for publication April 15, 1953.

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[1] On p. 1511 of the quoted paper, in the fourth line from the bottom, the absorption should have been stated in terms of "per day" instead of "per square millimeter."

TABLE 1. ABSORPTION RATES OF CORTICOIDS AND VARIOUS DERIVATIVES FROM 1,420 PELLETS OF THE PURE HORMONES

Steroids	No. of expts.	No. of pellets	Duration of expts. (days)	Total absorp. of group (μg.)	Total pellet surface* of group (sq. mm.)	Amt. absorbed per day and per sq. mm of the steroid hormone (μg.)
Progesterone	32	32	90	6,828	2,074	3.3±0.14
Desoxycorticosterone (DOC) acet.	18	18	90–93	3,147	1,166	2.7±0.09
17-Hydroxy-desoxycorticosterone (S) acet	30	78	90–91	13,988	5,054	2.8±0.05
Dehydrocorticosterone (A) acet.	8	8	42	3,306	411†	8.0±0.42
Dehydrocorticosterone (A) acet.	7	7	91	2,807	346†	6.0±0.35
Cortisone (E) acet.	21	32	42–48	13,640	2,044	6.7±0.12
17-Hydroxycorticosterone (F) acet.	23	36	43–47	4,870	2,333	2.1±0.13
17-Hydroxycorticosterone (F) acet.	23	36	90–92	4,292	2,333	1.8±0.10
Pregnenolone	23	254	90–94	10,218	16,459	0.6±0.01
Pregnenolone acet.	28	274	90–95	27,141	17,755	1.5±0.02
17-Hydroxy-pregnenolone	10	87	93–95	1,546	5,638	0.3±0.01
Δ^{16}-Dehydropregnenolone	14	216	90–91	9,092	13,997	0.7±0.01
Allo-pregnanolone	12	144	90	4,454	9,331	0.5±0.01
17-Hydroxy-allopregnanolone (L)	12	192	90–91	2,811	12,442	0.2±0.01
Δ^{11}-Dehydroprogesterone	6	6	91–92	610	389	1.6±0.08

* Surface of 1 pellet = 65 sq. mm.
† Four of these experiments with "½ pellet" only (38 sq. mm.).

Another very interesting feature is shown in Table 1, namely, the slow absorption of pregnenolone and its derivatives. Pregnenolone acetate has a higher absorption rate than free pregnenolone, but its absorption rate is still four to five times less than that of A or E. Remarkable also is the fact that whereas the substitution of $OH\cdot\cdot C_{17}$ in DOC (see S) does not change the absorption rate, the same substitution in pregnenolone and allopregnanolone considerably diminishes the absorption rate.

Results with mixed pellets containing 40 per cent of the respective steroid and 60 per cent of cholesterol are summarized in Table 2. As in the former paper (2), calculation was made on the assumption that the loss of weight of the pellet is due to nonselective absorption, *i.e.*, that both the specific steroid and cholesterol are absorbed, in such a way that the concentration of the former at the end of the experiment is the same as at the beginning. This has been shown to be the case for various steroid hormones (6–9) and it is assumed that the same applies also to other steroids when absorbed from mixed tablets.

As in our former work with progesterone and testosterone derivatives, the absorption rate *per square millimeter of the pellet surface* diminished as much as eleven (progesterone) to eighteen times (Kendall's compound

TABLE 2. ABSORPTION RATES FROM 230 MIXED PELLETS OF CORTICOIDS, CALCULATED ON THE ASSUMPTION OF *NONSELECTIVE* ABSORPTION OF THE STEROID HORMONES FROM THE MIXTURE, AND COMPARED TO ABSORPTION FROM PELLETS OF THE PURE HORMONE

Pellet containing 40% of:	No. of expts.	No. of pellets	Duration of expts. (days)	Total absorp. of group* (μg.)	Total pellet surface of group (sq. mm.)	Amt. absorbed per day and per sq. mm. of *pellet surf.* (μg.)	Amt. absorbed per day and per sq. mm. of *steroid horm. surf.* (μg.)		Relation of absorp. from pure (VIII) & from mixed (IX) pellets per sq. mm. of steroid horm. surf.
							pure pellet	40% of pellet†	
I	II	III	IV	V	VI	VII	VIII	IX	
Progesterone	13	13	90–92	285	842	0.3±0.05	3.3	0.8	1:0.24
DOC acet.	7	7	90	98	454	0.2±0.02	2.7	0.5	1:0.18
Reichst. comp. S acet.	13	19	90–91	224	1,231	0.2±0.01	2.6	0.5	1:0.19
Cortisone acet.	12	28	90–99	863	1,814	0.5±0.03	6.7	1.3	1:0.20
Kend. comp. F acet.	14	139	90–92	1,038	9,007	0.1±0.01	1.8	0.3	1:0.16
Δ^{11}-Dehydroprogesterone	12	24	91–92	263	1,555	0.2±0.01	1.6	1.5	1:0.31

* Forty per cent of loss of weight of pellets during the experiment.
† Figures of column VII multiplied by 2.5.

F); the absorption rate calculated *per square millimeter of the respective steroid hormone*, which occupied only 40 per cent of the pellet surface, diminished about four to six times.

However, one may reasonably argue that nonselective absorption has been demonstrated for certain steroids only, and that we do not know whether it applies to the corticoids in Table 2. To answer this argument, the whole loss of weight of the pellets has been tentatively assumed to be due to selective absorption of these corticoids, and in Table 3 the absorption rate has been calculated on the basis of this tentative assumption. As seen from Table 3, even on this assumption, the absorption rate per square millimeter of progesterone and of the four corticoids would be, with mixed pellets, 40 to 60 per cent less than with pellets containing the specific hormone only. The same applies to the interesting compound of Swiss workers, Δ^{11}-dehydroprogesterone (10).

The results as summarized in Table 3 are impressive: even when admitting selective absorption—a "generous" admission in view of the work with progesterone and various other steroids—absorption per square millimeter of the steroid hormone surface of a pellet diminished very considerably when cholesterol was added.

Another observation, though trivial and already made before by various authorities (11–14), may be mentioned here. For instance, when comparing the absorption rate calculated per day in experiments of forty-two and

TABLE 3. COMPARATIVE ABSORPTION RATES FROM PURE AND MIXED PELLETS OF CORTICOIDS, CALCULATED ON THE ASSUMPTION OF *SELECTIVE* ABSORPTION OF THE STEROID HORMONES FROM THE MIXTURE

	For comparison only: Absorbed per day and per sq. mm. of steroid horm. surf. of *pure pellet* (μg.) I	Total loss of wt. of mixed pellets of the group* (μg.) II	Surf. of steroid horm. in mixed pellets of the group† (sq. mm.) III	Amt. absorbed per day and per sq. mm. of steroid horm. surf. of *mixed pellets* (μg.) IV	Relation of absorp. from pure (I) and from mixed (IV) pellets per sq. mm. of steroid horm. surf.
Progesterone	*3.3*	713	337	*2.1*	1:0.64
DOC acet.	*2.7*	245	181	*1.4*	1:0.52
S acet.	*2.6*	560	492	*1.1*	1:0.42
Cortisone acet.	*6.7*	2,160	726	*3.0*	1:0.45
F acet.	*1.8*	2,590	3,603	*0.7*	1:0.39
Δ^{11}-Dehydroprogesterone	*1.6*	658	622	*1.1*	1:0.66

* Column V of Table 2, *multiplied* by 2.5.

† Column VI of Table 2, *divided* by 2.5. Figures of Column IV of Table 3 are thus 6.5 times greater than figures of Column VII of Table 2, but do not reach the level of the figures of the pure-pellet Column I.

ninety-one days' duration (A and F, Table 1) a smaller value is obtained in the longer experiments. This may be easily explained by the fact that in experiments of longer duration a considerable diminution of the pellet surface takes place and the total amount of absorption diminishes. However, calculation of absorption rates is perforce always made on the basis of the known pellet surface at the beginning of the experiment (see error 2 in (2)).

SUMMARY

Absorption rates of corticoids and their derivatives from subcutaneously implanted pellets, per square millimeter of pellet surface, are given.

The absorption rate of cortisone acetate is three to four times greater than that of 17-hydroxycorticosterone (Kendall's compound F) acetate.

Pregnenolone and its derivatives have the slowest rates. The absorption rate of pregnenolone is about ten times less, and that of pregnenolone acetate is four times less than the absorption rate of cortisone acetate.

The effect of cholesterol mixed with corticoids on the absorption rate of the latter is discussed. Full evidence is offered that the absorption rate *per square millimeter of corticoid* is considerably slowed down by the presence of cholesterol.

Acknowledgment

Thanks are due to Dr. A. White, Director of Research, and Dr. I. V. Sollins, Director, Chemical Specialties Co., New York, and Syntex, S. A., México, for generous supplies of compound S, pregnenolone and allo-pregnanolone, and their

derivatives; to Dr. R. T. Major, Scientific Director, and Dr. A. Gibson, Executive Director, Merck & Co., Rahway, N. J., for cortisone and compounds A and F; and to Dr. A. Wettstein, Vice-Director, Ciba, Basel, Switzerland, for Δ^{11}-dehydroprogesterone.

The pellets used in the study were from joint work with Dr. R. Iglesias, Dr. S. Bruzzone and Dr. Elvira Mardones, of this Department; cordial thanks are due to them for collaboration.

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PAPER CHROMATOGRAPHY OF PREGNANEDIOL AND RELATED COMPOUNDS*

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PREGNANE-3(α), 20(α)-diol has been isolated from urine on numerous occasions. Since the time it was first reported by Marrian (1), the compound has become known as pregnanediol. During pregnancy (2) or following the administration of progesterone (3) the urinary excretion of pregnanediol rises sharply and progesterone is therefore considered to be a precursor of this substance. Administration of 11-desoxycorticosterone has been found to increase pregnanediol excretion in animals (4), normal human subjects (5), and patients with Addison's disease (6), suggesting that pregnanediol is a metabolite of this corticoid. Although pregnane-3(α), 20(α)-diol seems to be the chief C_{21}-steroid alcohol found in pregnancy urine, it is by no means the only one, and the rise in pregnanediol excretion is known to be accompanied by increased excretion of allopregnane-3(α), 20(α)-diol and allopregnane-3(β), 20(α)-diol (7). In addition, several other chemically and possibly metabolically related C_{21}-steroids have been isolated from urine (8). The specificity of the methods used for the determination of pregnanediol in urine depends largely on the specificity of the isolation procedure. In the gravimetric determination of Venning (2) sodium pregnanediol glucuronidate is extracted from the urine with butanol and crystallized from aqueous acetone. However, the sodium pregnanediol glucuronidate thus obtained is not always homogeneous and it has been reported to contain as much as 23 per cent pregnane-3(α)-ol-20-one glucuronidate (8, 9). Similarly, methods of the type developed by Sommerville, Gough and Marrian (10) in which the urine is hydrolyzed with acid, extracted with toluene, the pregnane-3(α), 20(α)-diol precipitated,

Received for publication February 19, 1953.

* Supported by a contract between the Office of Naval Research, Department of the Navy, and the University of Minnesota (NR 114–020), and the Institutional Grant #49B of the American Cancer Society.

† American Scholar in Cancer Research, American Cancer Society.

and finally measured colorimetrically, may determine not only pregnane-3(α), 20(α)-diol but also allopregnanediols and other less well defined compounds. Perhaps one of the best indications of the difficulties attending the resolution of the pregnanediol fraction of urine is the observation of Mason and Kepler (11) that sodium pregnane-3(α), 17(α), 20(α)-triol glucuronidate and sodium pregnane-3(α), 20(α)-diol glucuronidate cannot be separated by fractional crystallization, and that mixtures of the unconjugated steroids have the melting point of pregnane-3(α), 20(α)-diol.

Our interest in the composition of the pregnanediol fraction was aroused by the finding of one of us (B. Z). that a considerable increase of pregnanediol-like materials occurs in the urine following either major surgical procedures or ACTH administration (12). In order to investigate the nature of these pregnanediol-like substances we have resorted to paper chromatography. The present communication deals with methods which we found useful for this purpose.

MATERIALS AND METHODS

We have studied the color reactions of nine C_{21}-steroids with antimony trichloride and zinc chloride, and their movement on propylene glycol-impregnated filter paper with toluene as the mobile phase (13) and on untreated filter paper with one of the ternary solvent systems recommended by Bush (14).

Unless otherwise stated, all chemicals used were of reagent grade. The sample of pregnane-3(α), 20(α)-diol was isolated by one of us (S.C.) from pooled samples of pregnancy urine; it had a melting point[1] of 240–1° C. Pregnane-3(α), 17(α), 20(α)-triol was also isolated in our laboratories (by S.C.) from the urine of an 11-year-old girl with the adrenogenital syndrome. The sample had a melting point[1] of 250–2° C. and its identity was verified by study of its infrared absorption spectrum in Dr. K. Dobriner's laboratory. We are greatly indebted to Dr. William H. Pearlman for sending us samples of the allopregnanediols, allopregnane-3(β)-ol-20-one and 5-pregnene-3(β)-ol-20-one. Progesterone and 17(α)-hydroxy-progesterone were commercially available.[2]

For the purpose of paper chromatography the steroids were dissolved in absolute ethanol in concentrations of 0.5 to 1.0 milligram per milliliter.

Use of the propylene glycol-toluene system: Sheets of Whatman #1 paper, 6×22½ inches in size, were dipped into a solution of 25 per cent propylene glycol in chloroform and dried in an air stream under a hood for one half hour. The steroid solutions were

[1] All melting points were determined on a Fisher melting-point block and are uncorrected.

[2] Glidden Company, Chicago, Ill.

then applied to a starting line five inches from the narrow margin of the paper by means of a micropipette. A total of from 5 to 25 micrograms of each compound was applied to the paper by repeated applications of the same solution if necessary. Care was taken to limit the maximum diameter of the spot to 1 centimeter, and the spots of various compounds or mixtures of compounds were spaced at least 3 centimeters apart. The papers were then placed in a cabinet[3] and developed with propylene glycol-saturated toluene by the descending method. The atmosphere inside the unit was kept saturated with toluene by placing dishes filled with the hydrocarbon at the bottom of the chamber. The papers were left in the cabinet for four to five hours, during which time the mobile phase descended to a distance of from 5 to 10 centimeters from the lower end of the strip. Equilibration and development were carried out at room temperature, which varied on different days over a range of 20° to 27° C. Following the development, the papers were first suspended under a hood and dried in an air draft, and then in an oven at 95° C. for half an hour. Following this final drying the color reagents were applied without delay.

Use of the water-methanol-toluene system (14): Bush's system B_4 was prepared by equilibrating a mixture of 1,000 milliliters of toluene, 500 milliliters of methanol, and 500 milliliters of water at 36° C. The mixture separated into two layers and the upper toluene layer was used for the development of the chromatograms. A glass cylinder, 18 inches high and 6 inches in diameter, furnished with a stand and trough for descending paper chromatography, was placed in a constant-temperature cabinet maintained at 36° C. Dishes filled with the upper and lower layers of the equilibrated water-methanol-toluene system were placed on the bottom of the cylinder and the atmosphere was allowed to become saturated with the solvent vapors at 36° C. The compounds were applied to strips of plain Whatman #1 filter paper, 3×16 inches in size, on a line 3 inches from a narrow margin. The papers were suspended from the trough and allowed to equilibrate with the solvent atmosphere overnight. Then the trough was filled with the upper layer of the water-methanol-toluene system. Within an hour, the mobile phase descended nearly the length of the paper. The strips were dried first in an air stream and finally *in vacuo* over phosphoric acid anhydride overnight. Color reagents were applied immediately following removal of the papers from the vacuum desiccator.

Color reaction with antimony trichloride in chloroform (15): A solution of antimony trichloride, saturated at room temperature was prepared. A wide flat glass dish was filled with enough of this solution to cover its bottom to a depth of about ½ centimeter, and the paper strip was rapidly drawn through the liquid. The reagent had to be used immediately after it had been poured into the dish, since it decomposed rapidly by absorption of water from the atmosphere. Immediately after the dipping, the paper was heated in an oven at 90° C. for four minutes. The colors produced by antimony trichloride tended to fade somewhat in the course of several days. The chromatograms could be stored as permanent records, but they had a low tensile strength and were very brittle.

Color reaction with zinc chloride in glacial acetic acid (16): A solution of 20 grams of zinc chloride in 30 milliliters of glacial acetic acid was prepared with gentle warming on a hot plate. The zinc chloride had been dried *in vacuo* over phosphoric acid anhydride and the reagent was freshly prepared immediately before use. It was poured into a flat dish and the paper drawn through it as described previously. The chromatograms were then heated in an oven at 95° C. for five minutes. Papers treated with zinc chloride

[3] Chromatocab B300—University Apparatus Company, Berkeley 3, California.

were very hygroscopic, and unless they were kept in a dry atmosphere, the chromatograms became moist and disintegrated.

The reactions with both antimony trichloride and zinc chloride would proceed only in the absence of water and alcohols. It was therefore necessary that the chromatograms be dried thoroughly. Also, the humidity of the atmosphere in which the dipping was carried out had to be kept low; a moisture content of about 70 grains of water per pound of air was about the upper limit of moisture content which we found compatible with the successful use of the antimony trichloride reagent.

RESULTS AND DISCUSSION

The color reactions of 8 of the 9 steroids studied and their mean R_f values in the two solvent systems are given in Table 1. Progesterone in

TABLE 1. COLOR REACTIONS AND R_f VALUES OF STEROIDS

Steroid	Color produced with				Solvent systems			
	Antimony trichloride		Zinc chloride		Propylene glycol-toluene		Water-methanol-toluene	
					R_f		R_f	
	in white light	in ult. viol. light	in white light	in ult. viol. light	Mean*	S.D.**	Mean*	S.D.**
Pregnane-3(α), 17(α), 20(α)-triol	violet	red	brown	blue	0.08	±0.02	0.44	±0.12
Allopregnane-3(β), 20(α)-diol	pink	pink	brown	blue	0.31	†	0.77	†
Pregnane-3(α), 20(α)-diol	purple	peach	brown	blue	0.32	±0.06	0.73	±0.07
Allopregnane-3(α), 20(α)-diol	pink	pink	brown	blue	0.35	±0.04	0.71	†
Allopregnane-3(β), 20(β)-diol	pink	pink	brown	blue	0.41	†	0.78	†
17(α)-Hydroxyprogesterone	none	blue	none	blue	0.59	±0.05	0.79	†
5-Pregnene-3(β)-ol-20-one	violet	red	pink	pink	0.78	±0.08	0.88	±0.03
Allopregnane-3(β)-ol-20-one	none	blue	none	none	0.86	†	0.89	†

* Means for 5-10 micrograms of steroid applied to paper
** Standard deviation.
† Mean of <5 determinations

amounts of 25 micrograms failed to yield a color with either antimony trichloride or zinc chloride, and it was therefore omitted from the table.[4] With antimony trichloride, the allopregnanediols produced a pink color in both white and ultraviolet light;[5] pregnane-3(α), 20(α)-diol gave colors which were very similar to those of the allopregnanediols but less intense; the colors produced by pregnane-3(α), 17(α), 20(α)-triol and 5-pregnene-3(β)-ol-20-one had a more bluish shade in white light and a more reddish shade in ultraviolet light. 17(α)-Hydroxyprogesterone and allopregnane-

[4] According to Neher and Wettstein (15) progesterone in amounts of 100 micrograms produces a faint violet color under ultraviolet light with antimony trichloride.

[5] Throughout these experiments a Black Light Products BL-2 lamp served as the source of ultraviolet light. It emitted a spectrum of 3,000 to 5,000 Å.

3(β)-ol-20-one produced colors in ultraviolet light only.[6] With zinc chloride, the allopregnanediols, pregnane-3(α), 20(α)-diol, and pregnane-3(α), 17(α), 20(α)-triol gave faint brown spots in white light and a blue fluorescence in ultraviolet light; no color was produced by allopregnane-3(β)-ol-20-one, and 17(α)-hydroxyprogesterone again gave a color only in ultraviolet light; in contrast to the other compounds in this series, 5-pregnene-3(β)-ol-20-one showed distinct pink coloration in both white and ultraviolet light.

The R_f values of the steroids were subject to considerable variations in either solvent system. They were computed as the quotient of the distance from the starting line to what was visually judged to be the middle of the spot in the direction of movement, over the distance from the starting line to the solvent front. The area of the spot on the chromatogram increased with the amount of steroid in the spot, and simultaneously the R_f values tended to decrease. For example, in a run designed to study the influence of the amount of pregnanediol in a spot on its R_f value in the propylene glycol-toluene system, spots containing 10, 20, 30, 40 and 50 micrograms of the steroid moved with R_f values of 0.36, 0.37, 0.34, 0.33 and 0.24 respectively. As might be expected, the temperature at which the chromatograms were developed also seemed to influence the R_f values; although we have not made any systematic observations on this point, it appeared that R_f values tended to decrease as the temperature decreased. Finally, in the propylene glycol-toluene system, the amount of propylene glycol used for impregnation of the paper affected the speed of movement of the mobile phase and the R_f values. The variability of the R_f values, although not desirable, actually did not prove to be much of a detriment to the separation and identification of the steroids, since, in any one run, the R_f values of all the compounds tended to vary in the same direction. The relative positions of individual steroids on the paper were thus much more constant than their R_f values. For instance, in four consecutive runs in which the pregnane-3(α), 20(α)-diol, the pregnane-3(α), 17(α), 20(α)-triol, and the three allopregnanediols were applied as individual spots, the order of movement was rather uniformly pregnane-3(α), 17(α), 20(α)-triol less than allopregnane-3(β), 20(α)-diol, less than pregnane-3(α), 20(α)-diol, less than allopregnane-3(α), 20(α)-diol, less than allopregnane-3(β), 20(β)-diol. Mixtures of the steroids could be resolved readily in all instances in which the range of R_f values of the components did not overlap appreciably. The resolving power of the propylene glycol-toluene system was therefore superior to that of the water-methanol-toluene system.

[6] Fifty micrograms of allopregnane-3(β)-ol-20-one were required to make the compound visible on the paper; even then, the spots were very faint.

Both systems separated mixtures of pregnane-3(α), 17(α), 20(α)-triol and pregnane-3(α), 20(α)-diol readily, but neither resolved mixtures of the allopregnanediols or of an allopregnanediol with pregnane-3(α), 20(α)-diol.

The methods just outlined have been used for a study of the pregnanediol fraction of female urine. From such studies it has been possible to demonstrate that substances chromatographically indistinguishable from both pregnane-3(α), 20(α)-diol and pregnane-3(α), 17(α), 20(α)-triol occur in the urine of normal women (17).

SUMMARY

Paper chromatographic techniques for the identification and separation of eight C_{21}-steroids likely to be present in the pregnanediol fraction of urine are discussed. Color reactions with antimony trichloride and zinc chloride, suitable for the detection of pregnane-3(α), 17(α), 20(α)-triol, pregnane-3(α), 20(α)-diol, allopregnane-3(α),20(α)-diol, allopregnane-3(β), 20(β)-diol, allopregnane-3(β), 20(α)-diol, 17(α)-hydroxyprogesterone, 5-pregnene-3(β)-ol-20-one, and allopregnane-3(β)-ol-20-one are reported. The movement of these compounds on paper in a propylene glycol-toluene system and a water-methanol-toluene system is described.

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ON THE CONVERSION OF INTRAVENOUSLY ADMINISTERED PROGESTERONE TO PREGNANEDIOL IN THE HUMAN FEMALE*

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INTRODUCTION

THE recently reported studies of West (1), and West, Tyler, Brown and Samuels (2) on the metabolism of intravenously administered testosterone have yielded results which emphasize the ideal character of the intravenous route for studies of this kind. Two recent events have given us a unique opportunity to study similarly the metabolism of progesterone. The first was the discovery (based on the reports of Bischoff and Pilhorn (3) and West (1)) that the preparation of any steroid for intravenous administration can be an exceedingly simple procedure (4). The other was the decision of this Department, based on this observation and on the report of Hertz *et al.* (5), to study the therapeutic effectiveness of intravenous progesterone in cervical carcinoma (6). The data reported here were obtained in conjunction with this work on carcinoma therapy, our main objective being the determination of the true conversion rate of progesterone to pregnanediol. As might be expected, our study raised more questions than it has answered, but it at least partially solved the problem that prompted its inception, and is interesting enough to warrant reporting now.

METHODS AND MATERIALS

Subjects. The subjects were 9 patients with carcinoma of the cervix and 9 relatively healthy young female subjects. Eight of the latter were inmates of a home for the feeble-minded,[1] and 1 was a private patient with

Received for publication November 29, 1952.

* Supported in part from Grants-in-Aid from the Ohio State Cancer Research Fund, and the Ohio State University Research Foundation.

[1] We wish to express our thanks to Miss Edythe Davis, matron of the Shady-Lawn Cottage, the Columbus State School, and to Drs. Walter Lowenstein and Roger Gove, medical director and superintendent, respectively, of the School, for their hearty cooperation and help in this study.

essential dysmenorrhea. The ages of the carcinoma patients ranged from 24 to 65 and those of the noncancerous subjects, from 18 to 25. All carcinoma patients were classified as League of Nations clinical class II or beyond. Physical examination of the noncancerous subjects showed no obvious abnormalities, and aside from the specific complaint of the private patient, all were judged to be in good health.

The noncancerous subjects were having regular menstrual cycles; body temperature curves were being kept on all of them for periods of time ranging from one to two years prior to the initiation of this particular study. All showed fairly regular biphasic curves. In each of these 9 cases, the administration of progesterone took place during the preovulatory portion of the cycle, as indicated from the previous menstrual history, the body temperature curve, and the low level of pregnanediol excreted before progesterone administration (all *pre-progesterone* urines assayed less than 0.30 mg. of pregnanediol per day).

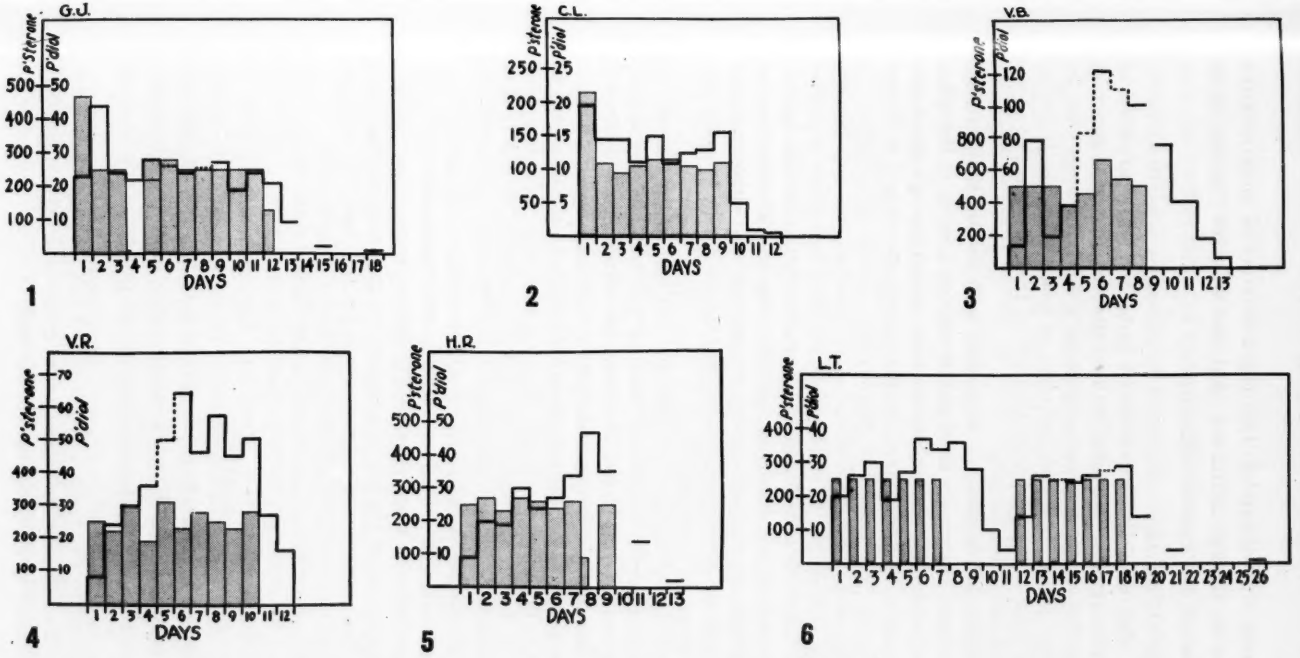
The menstrual histories of the cancerous patients are shown in Table 1.

TABLE 1. MENSTRUAL STATUS OF 9 PATIENTS WITH CERVICAL-CARCINOMA BEFORE TREATMENT WITH PROGESTERONE

Patient	Age (yrs.)	LMP* and/or previous menstrual history
V.B.	24	LMP 11 months before progesterone.
M.B.	29	LMP 3 years before progesterone. Cycles always very irregular, varying from 1–18 months in length.
S.S.	40	LMP 3 years before progesterone, following an abdominal operation of unknown character for unknown reasons.
V.R.	41	LMP less than 1 month before progesterone. Menstruation regular up to that time.
L.T.	42	Surgical menopause 6 years before progesterone.
H.R.	42	Surgical menopause 5 years before progesterone.
E.R.	47	Amenorrhea and hot flashes following x-irradiation 6 months before progesterone.
G.J.	61	Natural menopause 15 years before progesterone.
C.L.	64	Natural menopause 22 years before progesterone.

* Last menstrual period.

From this it can be seen that, with the possible exception of 1 patient (V.R., age 41), all were in a state of amenorrhea at the time of progesterone treatment. The amenorrhea was associated with the menopause (natural, surgical, or radiation) in 6, with the advanced state of the carcinoma in 1 (V.B., age 24), and with causes largely unknown in 1 (M.B., age 29). In the case of V.R., no accurate record of her last menstrual period before



FIGS. 1–6. *The day-to-day relation between the amount of progesterone administered and the excretion of pregnanediol.* In each of these graphs, the plan of presentation is the same. Progesterone dose and pregnanediol excretion are represented on the ordinate in milligrams, the progesterone by the shaded columns, and the pregnanediol by the connected vertical and horizontal lines. Note that in all cases the pregnanediol scale is 10 times that for progesterone, so that a ready appreciation of pregnanediol as a percentage of progesterone greater or less than 10 per cent can be obtained. The width of each column is used to represent the portion of each twenty-four hours in which progesterone was administered. Note that in the first 5 patients the administration was practically continuous, whereas in the sixth (L.T.) it occupied only twelve hours of each day. When, for whatever reason, pregnanediol determinations were not made on a particular day's urine, the estimated value for that day was determined by averaging the values of the two days on either side; such values are represented graphically by a broken line. It was felt, however, that in the period following progesterone administration such a procedure was not entirely justified. Blank places for certain days in some of the patients (days 14, 16 and 17 in G. J.; days 10 and 12 in H.R.; and days 20, 22, 23, 24 and 25 in L.T.) are thus days during the post-progesterone period on which no urines were assayed for pregnanediol.

progesterone treatment was obtained, but the low level of pregnanediol excretion previous to treatment indicated that she was very probably in the preovulatory phase of her cycle at this time.

Urine collections. Urine was collected from each of the carcinoma patients during her stay on the gynecology ward, and at various intervals during the follow-up period, at home. Collections were made in most cases on a 24-hour basis, the urine being preserved with thymol during the period of collection and refrigerated within a few hours after the completion of each 24-hour period. In several instances, as noted subsequently, collections were made on less than a 24-hour basis. Accuracy of the collection period was checked by means of creatinine determinations.

Techniques. Pregnanediol was determined on 10 per cent or 20 per cent aliquots of the 12-hour or 24-hour collections, and usually on 50 to 100 per cent aliquots of the collections of less than twelve hours. Most determinations were carried through on at least duplicate extracts of the urine; the final alcohol solution of each extract was in all cases assayed at least in duplicate. The method of Sommerville, Gough and Marrian (7), which measures total pregnanediol as the free steroid, after hydrolysis, was used throughout. Preceding this study, this method was thoroughly analyzed, and found to give recoveries of 80 per cent or better. In some instances urines were ultrafiltered through collodion (8) for the extraction of gonadotropins, and the pregnanediol determination carried out on the ultralfitrate, since we had previously shown (9) that although a small error may be associated with this procedure, the error was too small to be of significance in relation to the questions we were interested in.

Preparation of progesterone and dosages. Progesterone[2] was prepared for continuous intravenous administration (twelve or twenty-four hours per day) by adding the required volume of a propylene glycol (PG) solution of the hormone, containing either 10 or 20 mg. per cc., to a dilute solution of approximately 1,000 cc. of human serum albumin (HSA)[2] in 5 per cent dextrose in water. The amount of HSA in the total volume was determined by the amount of PG solution required. Thus, for each part of PG containing 10 mg./cc. of progesterone, 4 parts of concentrated HSA were required, or 9 to 10 parts if the PG solution contained 20 mg./cc. of progesterone (4). The total volume of 1,000 cc. was used simply for convenience in administration, this volume being used for any 12-hour period of administration. In several instances, as noted subsequently, a single intravenous injection was made. In these cases the PG-HSA mixture was not diluted, but administered as such. A dose of 100 mg. of progesterone thus was contained in 50 cc. of solution, of which 5 cc. was PG with progesterone 20 mg./cc., and 45 cc. was concentrated HSA. Other doses were carried in PG-HSA mixtures of the same ratio.

Specific doses and course of doses are noted subsequently, with the description of the results.

RESULTS

A. *The pattern of pregnanediol excretion during continuous and intermittent progesterone administration.* Five cervical carcinoma patients were treated with continuously administered intravenous progesterone in doses

[2] We are deeply indebted to Dr. Edward Henderson of the Schering Corporation, Bloomfield, N. J., and to the American Red Cross for the generous supplies of progesterone and human serum albumin, respectively, used in this study.

ranging from 100 to 500 mg./day for from nine to twelve days; 3 patients were treated with 250 mg./day, administered by continuous intravenous drip for twelve hours of each day for from seven to twelve days; 1 patient received 500 mg./day for ten days, the total dose being given intravenously over a period of one or two hours each morning. The patterns of progesterone administration, and of pregnanediol excretion during and following the period of progesterone administration in 7 of these patients, on whom complete studies were carried out, are shown in Figures 1–7. Since all pre-progesterone urines in this series of patients assayed less than 0.3 mg. of pregnanediol per day, only the post-progesterone excretion of pregnanediol is shown in these charts. Two general patterns of pregnanediol excretion are apparent. In one type (*i.e.*, Patients G.J. and C.L., Figs. 1 and 2) the daily pregnanediol excretion level is very constant, almost from the beginning; in the other type, (*i.e.*, Patients V.B., V.R. and H.R., Figs. 3, 4 and 5) the amount of pregananediol excreted each day steadily rises, but tends to reach a plateau roughly midway through the course of progesterone treatment. Though not strictly comparable with the others, because of the intermittency of progesterone administration, Patients L.T. and M.B. showed both types of excretion curve. Patient L.T. showed a rising curve during the first course of treatment, and a relatively flat one during the second course (Fig. 6), and Patient M.B. showed a very early plateau by the fourth day of treatment (Fig. 7).

B. *The independence of progesterone dosage and conversion percentage.* The range of progesterone doses used in this study, considering only single injections, or a single day's administration of progesterone (including the material from the section immediately following this one), was from 45 to 500 mg. If the doses of continuously administered progesterone are also included, the range extends up to 5,000 mg. The mean conversion percentage, considering only a single 24-hour period following progesterone administration in the non-continuous dosage group (45–500 mg. of progesterone) was 6.0, with a range of 3.9 to 9.1. In the continuous dosage group, which includes a dose range from 1,071 to 5,000 mg. (and *total* pregnanediol excretion), the mean conversion percentage was 13.5, with a range of 8.2 to 18.5. In both groups, the range was truly random, as shown by the straight-line character of the relationship between progesterone dosage and pregnanediol excretion (Fig. 8). The fact that the slopes of the two portions of the curves are almost parallel is an indication more of the difference in conversion percentages due to continuity of administration and completeness of pregnanediol recovery than it is of a direct relationship between dosage and excretion. It is of course, only another way of expressing the "lag" in conversion percentage that characterizes the early phase of

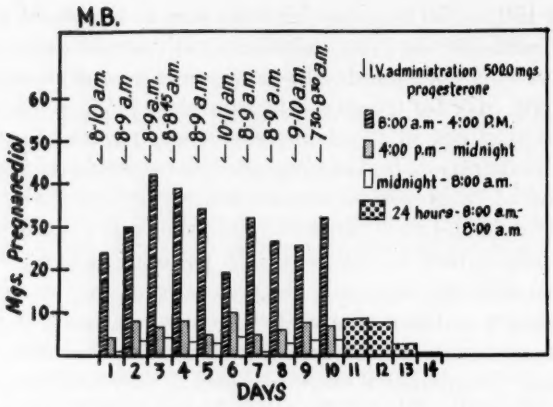


FIG. 7. *The excretion of pregnanediol in eight-hour periods in a patient who received daily injections of 500 mg. of progesterone in a period of one to two hours each day.* In this graph progesterone administration is shown by the half-arrows above each set of columns, and pregnanediol excretion by the height of the columns. In all instances, the three eight-hour periods of collection were from 8:00 A.M. to 4:00 P.M., from 4:00 P.M. to midnight, and from midnight to 8:00 A.M.. These are shown in the graph by differently shaded columns. Following the last administration of progesterone, urine was collected in 24-hour amounts for four days. For complete discussion of this and the preceding figures, see text.

continuous progesterone administration. This will be considered more fully in the discussion.

There are certain generalizations that may be made from the foregoing data, and from a detailed consideration of the excretion pattern of pregnanediol during the period of progesterone withdrawal. Inasmuch as the interest of these findings is primarily in their applicability to the "normal" individual, it is important, before going further, to examine the question of how "normal" these data are.

C. *A limited comparison of noncancerous and cancerous women.* Nine young, healthy, physiologically normal women (8 of them were feeble-minded and 1 of normal intelligence) were each given a single intravenous injection of progesterone. One received 43 mg. and one received 50 mg. on each of two occasions about a month apart; the remainder received 100 mg. All pre-injection urines assayed less than 0.3 mg. of pregnanediol per twenty-four hours. The total percentage of progesterone appearing as pregnanediol in twenty-four hours after the injection in these women is compared, in Figure 9, with the similar value in 9 women with cervical carcinoma. (The extra tenth value shown in Figure 9 for the cancerous patients is due to the fact that two conversion percentages were obtained from Patient V.B. The first was obtained from the course of treatment

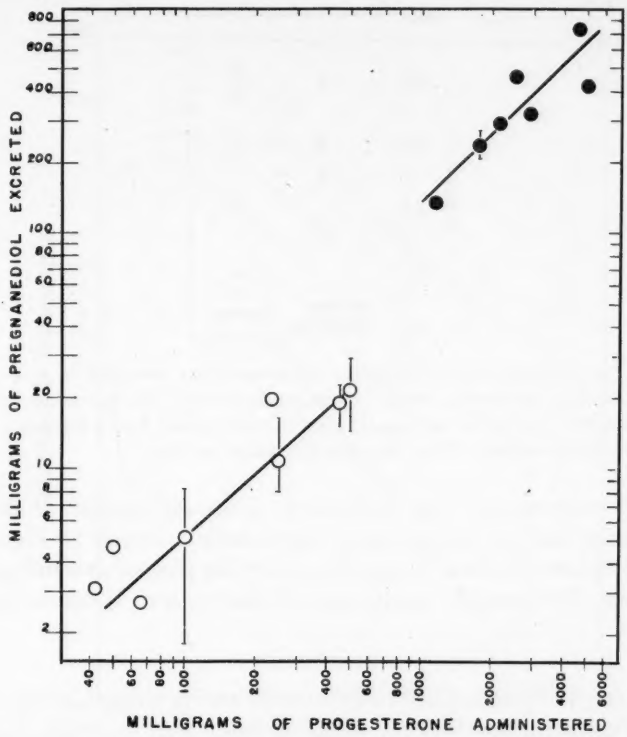


FIG. 8. *The relation between progesterone dose and pregnanediol excretion.* Pregnanediol is shown on the ordinate, progesterone on the abscissa. Open circles represent data from single progesterone injections, or a single day's administration of progesterone, and pregnanediol collected during only the period of twenty-four hours following or coincident with the injection. The closed circles represent data from continuously administered progesterone and complete pregnanediol collections, over periods of from nine to twelve days. For complete discussion, see text.

shown in Figure 3, and the second from another course of treatment, during which, except for the first day, incomplete urine collections were made. The two values were 2.8 per cent and 3.4 per cent, respectively.) The small difference between the means of the two sets of values is probably not significant. We make the assumption, based on these presented facts plus those already reported by others (5) concerning the effects of intramuscular injections, that there is no essential difference in progesterone conversion to pregnanediol between "normal" and "cancerous" women.

D. *The excretion of pregnanediol in the post-progesterone period.* As may be seen from Figures 1–7, the excretion of pregnanediol following the with-

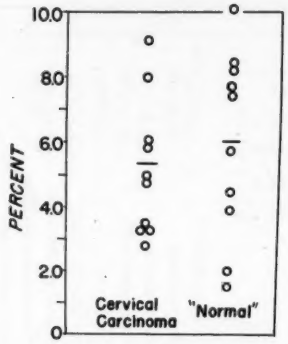


FIG. 9. *The similarity of the percentage of progesterone converted to pregnanediol in women with cervical carcinoma, and in noncancerous women.* The percentage of administered progesterone converted to pregnanediol in twenty-four hours following the injection is shown on the ordinate. For complete discussion, see text.

drawal of progesterone was suggestive of an asymptotic decline. That this excretion pattern was actually logarithmic is shown in Figure 10, in which the values obtained during this period are plotted as semilogarithmic coordinates. The possible significance of this pattern is considered in the discussion.

DISCUSSION

The pattern of pregnanediol excretion following the cessation of progesterone administration (Fig. 10) would indicate that excretion proceeds in exponential fashion over a rather long period of time. This pattern would be followed if progesterone conversion to pregnanediol were a first order reaction, and in addition (a) the metabolic rate of progesterone were relatively slow, with the resultant pregnanediol excreted as fast as it was formed, or (b) the metabolic rate of progesterone were so rapid that *all* of it were converted to pregnanediol and other metabolites practically immediately, and the pregnanediol excreted at a rate proportional to its concentration in the body fluids. It is also possible, in view of the studies of Sommerville and Marrian (10), that all the progesterone is converted to pregnanediol, and the latter metabolized at a rate proportional to its concentration and to such an extent that only 10 to 20 per cent of the amount originally formed appears in the urine.

It is obvious that these are only speculations, and that the data at hand do not permit of a choice between them; but it is also true that, since they must be considered as real possibilities related to the total process by which progesterone is metabolized, conversion percentages per se have only a limited significance. This does not mean that the studies indicating

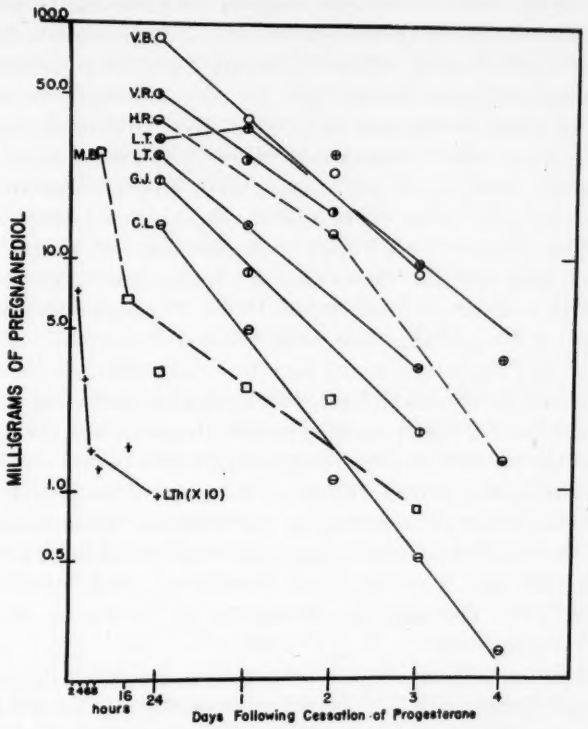


FIG. 10. *The pattern of pregnanediol excretion during the post-progesterone period.* The pregnanediol levels during the period following withdrawal of progesterone, shown individually in Figures 1–7, are here shown together as semilogarithmic coordinates, to bring out the exponential character of the decline. The pregnanediol excreted on the last day of progesterone administration is shown over the "24-hour" value of the abscissa, since this last day (which may be considered as day 0) was divided into hourly periods to bring out the changes in the excretion curves of Subjects L.Th. and M.B. Subject M.B. (Fig. 7) received ten daily injections of 500 mg. of progesterone, each dose being given during a period of one to two hours each morning. The 3 pregnanediol values shown here for the twenty-four hours of "day 0" are the means of the values for each of the ten days. Subject L.Th. received two injections of 50 mg. of progesterone on two separate occasions about a month apart, and collected urine at intervals of 2, 4, 6, 8, 16 and 24 hours on each of these days. The values shown here are the means of these two runs. For all of the subjects shown here a straight line has been drawn between connecting points whenever the points seemed reasonably fitted to a straight line. When there was more than a little doubt about such a fit, the points have been connected by a broken line. It should be obvious that only the *general* straight-line character of pregnanediol excretion in this period is being demonstrated. For complete discussion, see text.

differences in the conversion percentage of progesterone to pregnanediol in different physiologic or pathologic states (11–14) are not valid, but only that their true significance will not be realized until it is definitely known whether these conversion percentages are due to differences in the metabolic rates of progesterone and of pregnanediol, to differences in progesterone and pregnanediol distributions in the body fluids, or to the manner of pregnanediol excretion by the kidney. The two excretion curves of Subjects M.B. and L.Th. (Fig. 10) emphasize this point to some extent. Both these subjects received very rapid administrations of progesterone, and both showed pregnanediol excretion curves that are almost superimposable, although at different quantitative levels. The characteristic feature of these curves, distinguishing them from those of the remaining subjects in the series, is that the pregnanediol excretion falls off much more rapidly in the immediate eight to sixteen hours following administration of the progesterone than it does in the succeeding period. Regardless of the cause, it can be seen that the conversion percentage in the initial period is different from that in the succeeding period. Indeed, if the slope of the initial period were to be extended through a period of, for example, twenty-four hours, a much smaller total conversion percentage would have been obtained than was actually the case. The conversion percentage, thus, is at least in some way related to the processes which express themselves in the rate of excretion of pregnanediol.

The data presented here, however, permit a new interpretation of what has been called the "priming" effect of progesterone, an effect which we have preferred to designate as the "lag" in pregnanediol excretion.

Sommerville and Marrian (10) have seen a similar lag in individuals to whom progesterone was administered by the intramuscular or oral route, and have referred to it as the "priming" effect of progesterone. They have also presented data which they interpret as evidence that this priming effect is dependent on the presence of the uterus, since it was absent in men and in hysterectomized women. We take issue with this interpretation of the lag, however, on the following grounds. Our own data do not support the contention that the uterus is necessary for the occurrence of the "priming" effect. Of the 5 women who were given progesterone by continuous intravenous drip, 2 showed a very early plateau (G.J. and C.L.) of pregnanediol excretion, and each of these had an intact uterus (Fig. 1 and 2). Of the 3 who showed a late plateau, *i.e.*, the typical lag effect, 2 had intact uteri, but 1 had been supracervically hysterectomized several years prior to treatment (V.B., V.R. and H.R., respectively, Figs. 3, 4 and 5). Patients L.T. and M.B. are not included in this comparison because they received the progesterone intermittently, the former for twelve hours each day, and the latter for one hour each day; if included, these 2 patients

would also contradict Sommerville and Marrian's thesis (10). L.T. showed a typical lag during the first part of her progesterone administration (Fig. 6) although she was hysterectomized, whereas M.B. showed little lag, but had an intact uterus (Fig. 7). The objection might be raised that these patients were abnormal, inasmuch as they were suffering from carcinoma of the cervix; yet, since the argument pertains to the importance of the uterus in the manifestation of this lag, we must add the information that (a) the carcinoma was not in the uterus itself, and (b) had apparently not affected the uterus functionally, since all the women with intact uteri experienced typical withdrawal bleeding following the cessation of progesterone administration. This bleeding occurred in spite of the fact that most of them were menopausally amenorrheic (Table 1) and had not been pretreated with estrogens.

On what, then, does the lag phenomenon depend? We are fairly sure that although it very probably is characteristic of both endogenously secreted or intravenously administered progesterone, it is essentially a *short-lived* phenomenon. It is seen best of all in subjects receiving the hormone by intramuscular injection. Contrary to the views presented by Sommerville and Marrian (10), it is most likely in these subjects a reflection of the slower rate of absorption of the hormone from the intramuscular site, and not of a profound change in hormonal metabolism, such as might occur in the luteal phase of the cycle (12) or in pregnancy (12, 13). Sommerville and Marrian's (10) data actually support such a contention. In their cases there is, to our mind, a fairly clear difference between the intramuscular and the orally administered progesterone groups in the duration of the lag period. The oral group, in general, showed a definite tendency towards a curve with an early plateau, whereas *all* of the intramuscular group showed the opposite. This would be expected to happen solely on the basis of rapidity of absorption of the hormone. That the oral route is associated with relatively rapid absorption is shown by the fact that (as it did in our own series, in which there was no question of completeness of absorption) when progesterone administration was stopped, pregnanediol excretion fell off immediately, exponentially with time (our own calculations, based on their data), and at roughly the same rate as it did after intravenous administration of progesterone in our own series.

There is one last point which deserves mention. We must admit that the finding of a conversion percentage after intravenous progesterone which was essentially the same as that after intramuscular progesterone, was not expected. The reason for this was the reports concerning testosterone conversion to 17-ketosteroids, the one by Engstrom *et al.* (15) using intramuscular injections of testosterone propionate, and the other by West *et al.* (2) using intravenous testosterone. The former authors found about

35 per cent of the testosterone appearing as 17-ketosteroids in twenty-four hours following injection. The latter authors (as we have) found a similar percentage of the testosterone appearing as 17-ketosteroids within *two* hours following injection, and a total of 75 per cent in twenty-four hours. We have run some preliminary experiments (16) comparing intramuscular with intravenous *free* testosterone, which somewhat resolve this discrepancy. The free hormone is metabolized to 17-ketosteroids after intramuscular injection to the same *extent* as after intravenous injection, but a longer period of time is involved, and the decay curve is not as "clean" as it is after intravenous injection. The results of Engstrom *et al.* (15) are thus probably explained on the basis of the slower absorption of the hormone into the blood stream from the injection site, due to its esterification. In the case of progesterone, there is no difference in conversion percentage dependent on the route of administration, for the same reason that there is also none in the case of *free* testosterone.

SUMMARY

The amount of intravenously administered progesterone converted to pregnanediol in twenty-four hours following a single injection, or during the course of the first day of continuous administration, was of the order of 5 to 6 per cent, both in a group of 9 patients with cervical carcinoma and in a group of 9 noncancerous apparently healthy normal young women. This lack of difference in conversion percentages indicates the probability that the cervical carcinoma patients metabolized progesterone in a fairly normal manner, and validates the conclusions derivable from a more detailed study of progesterone metabolism in such patients.

During the course of continuous intravenous administration of progesterone in daily doses ranging from 100 to 500 mg. for from nine to twelve days, although pregnanediol excretion tended to rise during the early part of the administration it definitely showed a tendency to reach a plateau, even as early as the second day. Essentially, there was no difference in the conversion percentage of progesterone to pregnanediol in relation to the dose of progesterone, although the pregnanediol collected for twenty-four hours following a single injection or during a single day's administration of progesterone tended to be about half of all the pregnanediol collected following a course of continuous intravenous administration of progesterone.

Plotted as semilog coordinates, the excretion of pregnanediol during the period of progesterone withdrawal was a straight line with time, indicating the possibility that the conversion rate of progesterone to pregnanediol was a constant, of the nature of a first-order reaction.

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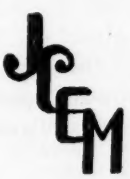

COMPARISON OF THE EFFECTS OF EPINEPHRINE AND NOR-EPINEPHRINE ON CIRCULATING EOSINOPHILS

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THE eosinopenia induced by epinephrine is well known, although the entire mechanism responsible for this effect is still not well understood. Factors other than a responsive adrenal cortex seem capable of evoking an eosinopenic response to epinephrine. The discovery of nor-epinephrine has been followed by several investigations of the ability of this compound to induce eosinopenia. Thorn *et al.* (1) have stated that nor-epinephrine is practically devoid of eosinolytic effect. The results of several investigations of the comparative eosinolytic effects of epinephrine and nor-epinephrine in normal fasting human subjects and in the mouse indicate that the latter is approximately one-sixth as potent as the former in this respect (2–5).

This study was undertaken to confirm and extend previous investigations of the relative eosinolytic properties of epinephrine and nor-epinephrine, with special reference to their effect upon patients with disorders not involving endocrine organs and in patients with impaired adrenocortical and anterior pituitary function.

Our subjects were studied in the postabsorptive state and were given only water from at least twelve hours before the test until after the last eosinophil count, four hours after the injection. Moderate physical activity was allowed during this four-hour interval. Eosinophil counts were made on venous blood immediately after withdrawal, employing the standard technique and a counting chamber of the Levy type. An interval of at least three days was allowed between successive studies in each patient.

In patients receiving cortisone this medication was discontinued for three days before each study, but three of our patients with Addison's disease were still presumably absorbing desoxycorticosterone from subcutaneously implanted pellets at the time of their studies.

Our patients were divided into four groups as follows:

Group 1. Ten patients with miscellaneous non-endocrine disorders in

Received for publication March 12, 1953.

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whom eosinophil counts were made before and at hourly intervals for four hours following the subcutaneous injection of 0.15 mg. of nor-epinephrine.[1]

Group 2a. Five patients with miscellaneous non-endocrine disorders in whom eosinophil counts were made before, and four hours after the subcutaneous injection of 0.3 mg. of nor-epinephrine. Similar counts were made in these patients after intervals of not less than three days, following a subcutaneous injection of 0.3 mg. of epinephrine.[2]

Group 2b. Five patients with miscellaneous non-endocrine disorders in whom procedures identical with those described in Group 2a were carried out. In addition, eosinophil counts were made before, and four hours after the subcutaneous injection of 0.3 cc. of distilled water in these patients.

Group 3. Five patients with primary adrenocortical deficiency and 1 patient with equivocal evidence of primary adrenocortical deficiency, in whom procedures identical with those described in Group 2b were carried out.

Group 4. Three patients with anterior pituitary deficiency in whom procedures identical with those described in Group 2b were carried out.

In Groups 2a, 2b, 3 and 4, the order in which the respective injections of nor-epinephrine, epinephrine and distilled water were given, was varied. The results of our observations are presented in Tables 1, 2, 3 and 4.

In Group 1, the maximal change in eosinophil count during the first four hours after the subcutaneous injection of 0.15 mg. of nor-epinephrine varied from +8 per cent to −59 per cent. In only 1 instance did a decrease of more than 50 per cent occur. The mean change was −28 per cent. Among the 8 patients who showed a decrease, the maximum fall was noted at the end of four hours in 4 and at one to two hours in 3.

In Groups 2a and 2b the mean variation in eosinophil count following the injection of 0.3 mg. of epinephrine was −60.2 per cent, whereas similar values following the injection of 0.3 mg. of nor-epinephrine and 0.3 cc. of distilled water were −22 per cent and −0.7 per cent, respectively. In Group 2b the difference in response to water and to nor-epinephrine was not statistically significant. In Groups 2a and 2b, however, there were significant differences in response to epinephrine and to nor-epinephrine or to distilled water. The difference in response to 0.15 mg. of nor-epinephrine (Group 1) and to 0.3 mg. of the same compound (Groups 2a and 2b) was not significant.

In Group 3 the mean variations among the 5 patients with unquestioned

[1] Levophed (levo-arterenol bitartrate monohydrate) was supplied by Winthrop Stearns, Inc., New York.

[2] Supranephrin (*l*-epinephrine hydrochloride), William Rorer, Inc., Philadelphia, Pa., containing from 10 to 18 per cent of nor-epinephrine.

TABLE 1. RESPONSE OF CIRCULATING EOSINOPHILS TO SUBCUTANEOUS INJECTION OF 0.15 MG. OF NOR-EPINEPHRINE IN 10 PATIENTS WITH MISCELLANEOUS NON-ENDOCRINE DISORDERS

Name	Diagnosis	Eosinophil count per cu. mm. in venous blood (fasting)	Percentile change in circulating eosinophils			
			1st hour	2nd hour	3rd hour	4th hour
R.T.	Duodenal ulcer	200	+ 9.0	− 9.0	− 5.5	−19.0
C.M.	Duodenal ulcer	490	−20.5	+ 8.3	− 3.7	−19.4
L.F.	Benign essential hypertension	72	+ 8.3	− 4.2	− 4.2	−57.0
S.M.	Peripheral vascular disease	130	− 1.6	+ 7.7	+20.7	+ 8.4
J.M.	Inguinal hernia	150	−24.4	−16.7	−50.0	−48.0
D.M.	Anxiety neurosis	25	+24.0	+12.0	− 4.0	−36.0
M.S.	Idiopathic epilepsy	200	+ 5.0	−35	− 2.5	−31.0
G.K.	Thrombophlebitis	319	− 6.0	−16.3	−30.5	−42.0
P.P.	Pulmonary emphysema	94	− 3.2	− 3.2	+ 9.5	+ 6.1
E.G.	Benign essential hypertension	50	−38.0	−50.0	−48	−46
Mean percentile change:			*− 4.7*	*− 7.4*	*−11.8*	*−28.3*

primary adrenocortical deficiency were as follows: after 0.3 mg. of epinephrine, −5.6 per cent; after 0.3 mg. of nor-epinephrine, +8.7 per cent; and after 0.3 cc. of distilled water, −7.3 per cent. If the sixth patient (with inactive pulmonary tuberculosis and equivocal evidence of adrenocortical deficiency) is included, the mean variations are as follows: after epinephrine, −7.8 per cent; after nor-epinephrine, +6 per cent; and after water, −8.4 per cent.

In Group 4 the mean variations were as follows: after 0.3 mg. of epinephrine, −16 per cent; after 0.3 mg. of nor-epinephrine, +5.8 per cent; and after 0.3 cc. of distilled water, −1.7 per cent. In Groups 3 and 4 the differences in response to epinephrine, nor-epinephrine and distilled water were not significant.

The results of these observations are in general agreement with previous reports on the lack of effect of nor-epinephrine in inducing eosinopenia in normal human subjects and laboratory animals.

Humphreys (2) found that among 24 medical students, a mean variation of −48 per cent occurred in circulating eosinophils following the stress

TABLE 2. RESPONSE OF CIRCULATING EOSINOPHILS TO SUBCUTANEOUS INJECTION OF 0.3 MG. OF EPINEPHRINE, 0.3 MG. OF NOR-EPINEPHRINE AND (IN 5 PATIENTS) 0.3 CC. OF DISTILLED WATER IN 10 PATIENTS WITH MISCELLANEOUS NON-ENDOCRINE DISORDERS

Name	Diagnosis	Epinephrine (0.3 mg.)		Nor-epinephrine (0.3 mg.)		Distilled water (0.3 cc.)	
		Eosinophil count per cu mm. (fasting)	Percentile change in circ'g. eosinophils	Eosinophil count per cu. mm. (fasting)	Percentile change in circ'g. eosinophils	Eosinophil count per cu. mm. (fasting)	Percentile change in circ'g. eosinophils
J.K.	Gastric ulcer; thrombophlebitis*	496	−16.8	722	− 7.8		
A.C.	Functional amenorrhea	32	−81.3	44	−43.6		
M.K	Euthyroid state for 1 year after I^{131} treatment	106	−51.0	119	−39.5		
R.H.	Optic atrophy	284	−62.6	306	−27.0		
E.C.	Post-traumatic cranial exostosis	253	−56.0	276	−46.0		
J.G.B.	Inguinal hernia	344	−85.2	219	−10.5	381	+3.4
W.B.	Dwarfism (nutritional)	131	−57.3	191	−25.6	106	0.0
M.M.	Chronic pes planus	178	−58	161	− 5.0	175	−7.5
S.S.	Anorexia nervosa	144	−65.3	130	−14.0	144	−9.8
M.P.	Atherosclerosis	115	−68.0	118	0.0	109	−7.0
Mean percentile change:			*−60.1*		*−21.9*		*−1.4*

* This patient had acute pain due to thrombophlebitis of the leg before the injection of epinephrine It is interesting that his initial count on this occasion was 33 per cent less than the initial count prior to the injection of nor-epinephrine three days previously.

induced by taking term examinations. Epinephrine and nor-epinephrine, in doses of 0.25 mg. each, caused variations of −61 per cent and −9 per cent respectively in the same subjects plus a group of 12 patients with non-endocrine disorders. Madison (3), using 0.3 mg. each of epinephrine and

TABLE 3. RESPONSE OF CIRCULATING EOSINOPHILS TO SUBCUTANEOUS INJECTION OF 0.3 MG. OF EPINEPHRINE, 0.3 MG. OF NOR-EPINEPHRINE AND 0.3 CC. OF DISTILLED WATER IN 5 PATIENTS WITH PRIMARY ADRENOCORTICAL DEFICIENCY AND 1 PATIENT WITH EQUIVOCAL EVIDENCE OF ADRENOCORTICAL DEFICIENCY

Name	Diagnosis	Epinephrine (0.3 mg.)		Nor-epinephrine (0.3 mg.)		Distilled water (0.3 cc.)	
		Eosinophil count per cu. mm. (fasting)	Percentile change in circ'g. eosinophils	Eosinohpil count per cu. mm. (fasting)	Percentile change in circ'g. eosinophils	Eosinophil count per cu. mm. (fasting)	Percentile change in circ'g. eosinophils
T.S.	Addison's disease	341	+22.8	634	+ 7.5	717	−18.5
L.S.	Addison's disease	225	−22.3	287	0.0	172	−10.0
H.M.	Addison's disease	100	0.0	116	+ 3.4	122	−10.7
J.J.	Addison's disease	131	− 9.2	114	+17.3	150	− 4.0
N.H.	Addison's disease	116	−19.0	119	+15.5	125	+ 6.4
A.F.	Inactive pulmonary tuberculosis; ?Addison's	200	−19.0	206	− 7.3	190	−13.7
Mean percentile change:			*− 7.8*		*+ 2.7*		*− 8.4*

TABLE 4. RESPONSE OF CIRCULATING EOSINOPHILS TO SUBCUTANEOUS INJECTION OF 0.3 MG. OF EPINEPHRINE, 0.3 MG. OF NOR-EPINEPHRINE AND 0.3 CC. OF DISTILLED WATER IN 3 PATIENTS WITH HYPOPITUITARISM

Name	Diagnosis	Epinephrine (0.3 mg.)		Nor-epinephrine (0.3 mg.)		Distilled water (0.3 cc.)	
		Eosinophil count per cu. mm. (fasting)	Percentile change in circ'g. eosinophils	Eosinophil count per cu. mm. (fasting)	Percentile change in circ'g. eosinophils	Eosinophil count per cu. mm. (fasting)	Percentile change in circ'g. eosinophils
H.R	Craniopharyngioma	267	−15.7	262	− 3.5	231	−5.2
J.L.	Panhypopituitarism	275	−17.1	275	+31.6	312	0.0
H.S.	Craniopharyngioma	118	−15.3	112	−10.8	106	0.0
Mean percentile change			−16		+ 5.4		−1.7

nor-epinephrine and 0.3 cc. of normal saline in 15 normal human subjects, reported mean variations of −54.9 per cent, −8.2 per cent and −8.3 per cent, respectively. Pellegrino *et al.* (4) administered epinephrine and nor-epinephrine intravenously, giving 0.2 and 0.3 mg. of each respectively in 200 cc. of normal saline solution over a sixty-minute period to 53 non-endocrine patients. The larger dose of epinephrine produced a mean variation in circulating eosinophils of −48.6 per cent; the smaller dose, a mean variation of −35 per cent. They stated that similar doses of nor-epinephrine were not followed by a significant decrease in eosinophils. Scott (5) found that in mice, 0.06 mg. of nor-epinephrine was required to produce an eosinopenia comparable to that which followed injection of 0.01 mg. of epinephrine.

Our observations in 20 patients with non-endocrine disorders indicate that, although nor-epinephrine produced some decrease in circulating eosinophils both in doses of 0.15 mg. and 0.3 mg., this decrease was significantly less than that produced by epinephrine in doses of 0.3 mg. In 5 of these patients there was no statistically significant difference between the decrease in eosinophils which followed the subcutaneous injection of 0.3 mg. of nor-epinephrine and that which followed 0.3 cc. of distilled water.

In our patients with primary adrenocortical deficiency and hypopituitarism, neither epinephrine, nor-epinephrine nor distilled water produced a significant decrease in circulating eosinophils.

Present evidence suggests that, under normal conditions, epinephrine is not an important factor in stimulating the release of adrenocorticotropin (6), and that other mechanisms, still obscure, are also capable of an eosinolytic effect following the injection of epinephrine. Thus there is some evidence to suggest that such eosinopenia may be due to the peripheral synergistic action of epinephrine with the 11-17 oxysteroids of the adrenal

cortex (7). Whatever these mechanisms may be, it would appear from our observations as well as from those previously reported that nor-epinephrine is significantly less potent than epinephrine in initiating them or in evoking an eosinolytic adrenocortical response. It would also appear that, in the presence of both primary and secondary adrenocortical deficiency, nor-epinephrine is no more capable than epinephrine of inducing eosinopenia.

SUMMARY

1. Changes in the number of circulating eosinophils at intervals up to four hours following the subcutaneous injection of epinephrine, nor-epinephrine and distilled water have been determined in 20 patients with miscellaneous non-endocrine disorders. Similar observations have been made in 5 patients with primary adrenocortical deficiency, in 1 with equivocal evidence of primary adrenocortical deficiency and in 3 patients with hypopituitarism.

2. The results indicate that in patients without apparent endocrine disorder the eosinopenia following the administration of epinephrine is significantly greater than that induced by nor-epinephrine.

In patients with adrenocortical or pituitary deficiency both epinephrine and nor-epinephrine failed to produce a significant decrease in circulating eosinophils under the conditions of the test.

Acknowledgment

The author is indebted to Doctors Isaac Starr and Edward Rose for suggestions and advice, and to Mrs. Laurette Joline for technical assistance.

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THE EVOLUTION OF NODULAR GOITER

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SIMPLE goiter occurs sporadically all over the world and in the form of endemic goiter affects millions of people in every continent, as a recent World Health Organization conference[1] so clearly showed. Simple goiter almost inevitably progresses to the nodular form, and thus a clear knowledge of how these nodules arise and what changes they may undergo is of importance to the doctor, whether he is working in office, hospital, laboratory or public health department. Nodular goiter may become the seat of hyperthyroidism. When this occurs, Plummer (1, 2) and others have stressed that the clinical picture is different from that seen in the younger patient with the non-nodular gland of Graves' disease. Finally, the quickening of interest in thyroid cancer and its possible association with nodular goiter makes a study of the latter mandatory.

This particular investigation started with an attempt to calculate more accurately the amount of irradiation received by the thyroid when radioactive iodine is administered therapeutically. This necessitated taking into account the patchy uptake so often seen in autoradiographs prepared from excised portions of the gland. For this purpose patients with nontoxic goiter who were to undergo thyroidectomy were given a tracer dose of radioactive iodine (100 microcuries of I^{131}) and, after operation, autoradiographs were prepared from sections made through whole lobes. It was soon apparent that the geometry of blackening on the film, and therefore of iodine uptake, was not capable of reliable mathematical interpretation. However, comparison of histologic pattern and iodine uptake showed that those areas where blackening of the autoradiograph occurred could be correlated with definite characteristics in the follicles, which could usually be measured.

There is a steady flow of patients with nontoxic goiter through the clinic and a proportion of these require thyroidectomy for various reasons—usually, because of symptoms due to pressure on the trachea, great vessels or esophagus; occasionally, when the possibility of a malignant change

Received for publication April 16, 1953.

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† In receipt of a grant from the Medical Research Council.

[1] W. H. O. Conference on Endemic Goiter, London, England, December, 1952.

cannot be excluded by clinical investigations; and less often, for cosmetic reasons. More than 60 thyroid glands have been investigated by the technique described and the results form the basis of this communication.

METHOD

A careful case history was obtained from each patient, due regard being given to the incidence of goiter in other members of the family and in the district where the patient lived. Enquiries were made about diet, but no close correlation was discovered between the incidence of goiter and any particular article of food (cabbage is mentioned subsequently). The neck and chest were examined by x-ray and a complete blood count made. The patient then received 100 microcuries of I^{131} after a brief fast. The urine was collected in three periods over the next forty-eight hours and its radioactive content measured in a multi-tube fluid counter, after the method of Arnott *et al.* (3). This gives a good index of total thyroid function. Between thirty-six and forty-eight hours after the tracer dose, the neck was scanned with a collimated counter (Veall (4)) and the iodine chart so obtained (Taylor and Stewart (5)) showed if there were any obvious areas of diminished or increased iodine uptake in the gland. After forty-eight hours the patient was submitted to thyroidectomy, the amount of tissue removed being determined by the size of the gland, the age of the patient and the over-all radioactive iodine uptake as measured by urinary excretion.

The excised thyroid was weighed and a drawing made. Slices about 0.25 cm. thick were cut through whole lobes in the planes thought most likely to yield information. These slices of tissue were fixed in 10 per cent formol saline, embedded in paraffin wax and cut on a sledge microtome. The technique was not always easy, as the consistency of nodular goiter is usually variable and calcification is often encountered. The sections, after mounting and clearing, were placed in close contact with fine grain x-ray film and stored in light-tight boxes in a refrigerator. Samples were removed at one, two, and eight days and developed. The sections were then stained with hematoxylin and eosin and compared with the autoradiographs. When it was found necessary to measure the diameters of many follicles, a simple apparatus embodying a projection microscope and a fine-grain translucent screen (Taylor (6)) was employed.

RESULTS

It is unlikely that the development of a goiter will ever be demonstrated by serial biopsies of a thyroid gland over a space of years. Indeed no biopsy of this gland can be considered representative of the whole. From a series of glands such as those studied here, however, it is possible to reconstruct the various stages in the evolution of a nodular goiter, although it is admitted that other interpretations are possible.

When the histologic and autoradiographic evidence presented by these glands is considered in conjunction with the age of the individual, the duration of symptoms, and the geographic background (*i.e.*, whether the district is one in which endemic goiter is found), it is possible to group the goiters in a series. This series begins with a simple diffuse enlargement of the gland in a young patient and ends with the large multinodular goiter showing evidence of hemorrhage and calcification, in the older individual. It

is stressed that none of these patients showed evidence of hyperthyroidism or malignant disease.

For convenience, a brief case history of a single representative from each group is given, together with a photomicrograph and autoradiograph prepared from a section made through a whole lobe of the gland or all of the excised tissue.

Group 1. Simple diffuse enlargement of the thyroid (Fig. 1)

It is unusual for a thyroidectomy to be necessary in this condition, but it was called for on three occasions in which a young patient (average age 25; 2 males and 1 female) was distressed by the increasing size of the neck. The glands showed no nodules clinically or on naked-eye inspection of the cut surface. The urinary radioiodine excretion values were low, showing that the gland retained more of the isotope than normally, though the pattern was not typically that of hyperthyroidism. All 3 patients came from Southern Ireland where there are areas of endemic goiter.

Group 2a. Areas of local hyperfunction (Figs. 2 and 3)

Many thyroids showed small discrete areas of hyperfunction as indicated by blackening on the autoradiographs. In these areas, follicles of small diameter were grouped together and their significance is considered in the discussion later. Such areas were seen in every form, from mere clusters of follicles to a clearly defined nodule. When small, they were always multiple and the intervening areas were relatively inactive. These hyperfunctioning areas were more liberally supplied with blood vessels than the remainder of the gland.

Group 2b. The solitary hyperactive nodule (Fig. 4)

This finding was rare and there were only 2 examples of it. Both patients showed a urinary excretion of radioactive iodine on the low side of normal, suggesting overactivity of the gland, but the findings were within the range considered normal for this region. It would appear that all of the patient's thyroid hormone was being manufactured in this solitary area and apparently, despite the intense activity, the mass of tissue was not sufficient to cause hyperthyroidism. This group appears to form a close link with those hyperthyroid patients who show a solitary toxic nodule, described clinically by Plummer (2) and confirmed with the use of radioiodine techniques by Cope (7).

Group 3. Hyperactive nodule showing hemorrhage and necrosis (Fig. 5)

Each nodule in the thyroid gland appears to be created as a focus of intense activity and this very activity leads to its own ultimate destruc-

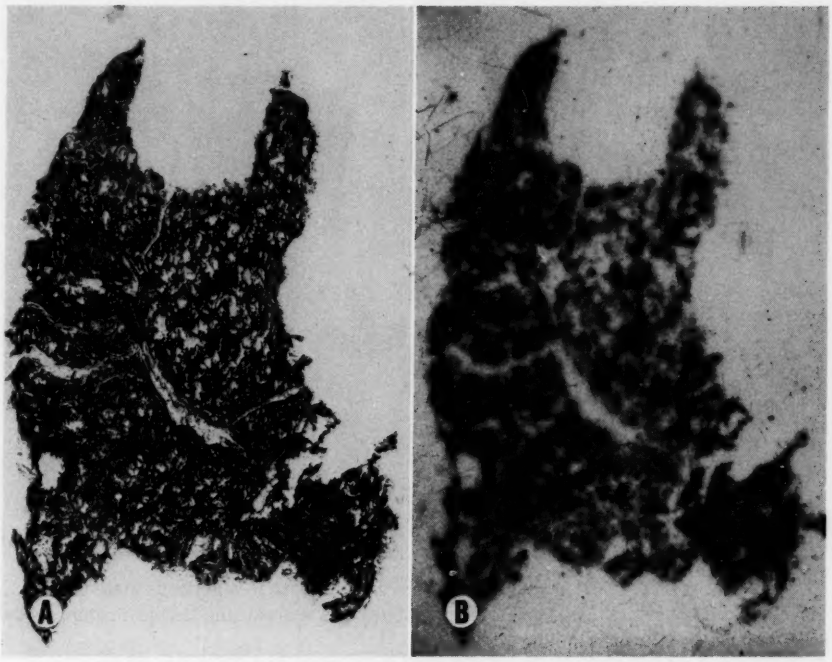

FIG. 1. *H. H. No. 125207.* A single male barber, aged 22, was born and bred in Ireland in an area of endemic goiter and lived on a diet rich in cabbage and turnips. The goiter was first noticed when he was 20, He left Ireland five months later, and his neck continued to enlarge.

Thyroid. Symmetrically enlarged, smooth, and about five times normal size; thrill and bruit detected over both lobes.

Serum cholesterol level 192 mg./100 ml. B.M.R.'s, −10 and −6 per cent. Radioactive iodine excretion test:

0– 8 hours,	6.3 per cent
8–24 hours,	0.6 per cent
24–48 hours,	0.3 per cent
Total	7.2 per cent in 48 hours

The patient was treated with desiccated thyroid for six months, with slight diminution in the size of the goiter. Thyroid was omitted six months before operation.

Operation. Partial thyroidectomy.

(A) *Pathology.* Fairly uniform overall appearance with many follicles showing well marked activity; a few show flattened epithelium and are distended with colloid.

(B) *Autoradiograph.* Radioactive iodine uptake is uniformly spread throughout the whole section.

tion, for the increased vascularity culminates in hemorrhage and necrosis in the center of the nodule. That this destruction is of fairly sudden onset is shown in the accompanying autoradiograph, in which it will be seen

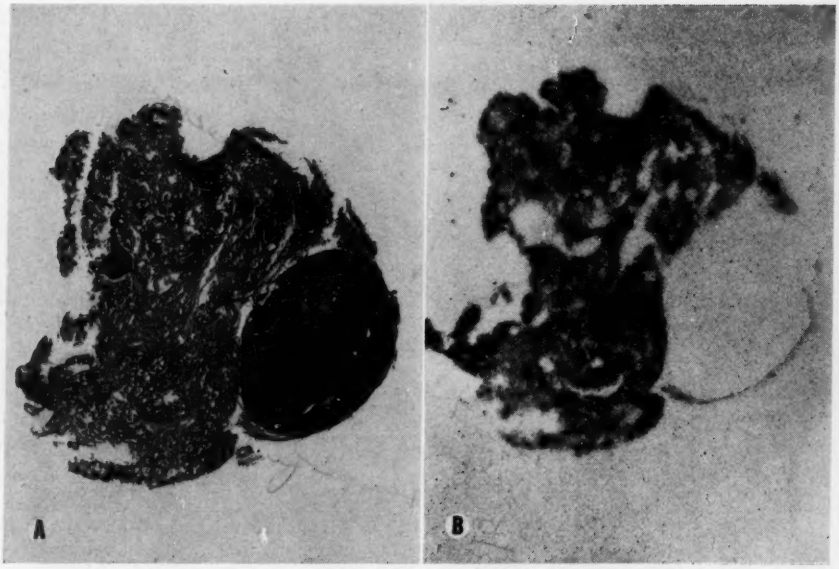

FIG. 2. *H. H. No. 27107.* A housewife, aged 41, was born in a goitrous area of Ireland. A swelling of the neck which she had had for ten years was causing tracheal compression. She slept propped up with pillows.

Thyroid. Symmetrically enlarged to about five times normal size.

Radioactive iodine excretion test:

0– 8 hours,	33.3 per cent
8–24 hours,	9.5 per cent
24–48 hours,	0.9 per cent
Total	43.7 per cent in 48 hours

Operation. Partial thyroidectomy.

(A) *Pathology.* Two diffusely enlarged thyroid lobes showing large inactive acini filled with solid eosinophilic colloid. There are a few discrete groups of small follicles with tall cells showing activity. The solid nodule is a large clear-cell adenoma, mostly trabecular, with a few small follicles (so-called fetal adenoma).

(B) *Autoradiograph.* Practically no radioactive iodine uptake, except in two tiny foci which correspond to areas of small follicles with increased cell height. The solid nodule (fetal adenoma) shows no radioactive iodine uptake.

that the nodule still had a rind of active follicles capable of taking up radioiodine, a phenomenon first described by Dobyns and Lennon (8).

Group 4. Inactive nodules (Figs. 6 and 7)

As time passes, more and more of the nodules in the gland are found to be incapable of taking up radioiodine. These inactive nodules show widely varied histologic structure. They may be made up of follicles of varying

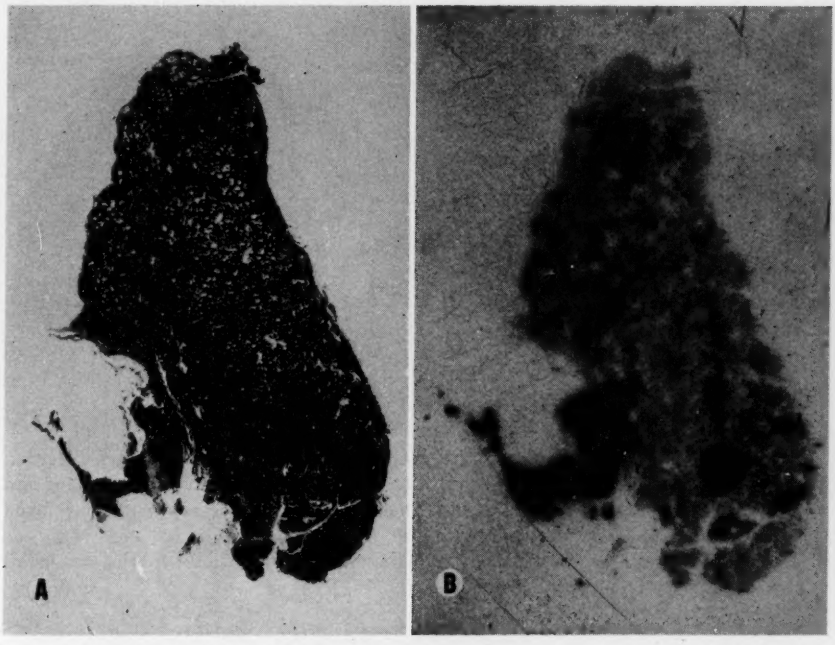

FIG. 3. *H. H. No. 131977.* A housewife, aged 35, was born and bred in West London, and had a swelling in the right side of her neck for five years. She suffered from palpitation, nervousness and flatulence and was emotionally unstable.

Thyroid. Readily palpable, with a small nodule in the right lobe.

Radioactive iodine excretion test:

0– 8 hours,	29.1 per cent
8–24 hours,	8.4 per cent
24–48 hours,	2.5 per cent
Total	40.0 per cent in 48 hours

Operation. Partial thyroidectomy.

(A) *Pathology.* Uniformly firm thyroid, with a soft hemorrhagic nodule 1.5 cm. in diameter. Main mass of thyroid tissue shows inactive follicles lined by low epithelium. The nodule and a second smaller nodule show small follicles with tall cells typical of increased activity.

(B) *Autoradiograph.* No appreciable radioactive iodine uptake except in two areas which histologically are composed of small follicles with tall cells.

diameters, but nearly all are lined by flattened or low cuboidal epithelium. Occasionally the whole nodule is replaced by a single lake of colloid. Others appear as true cysts lined by fibrous tissue; calcification is not uncommon and presumably follows hemorrhage. When it is considered how rapidly

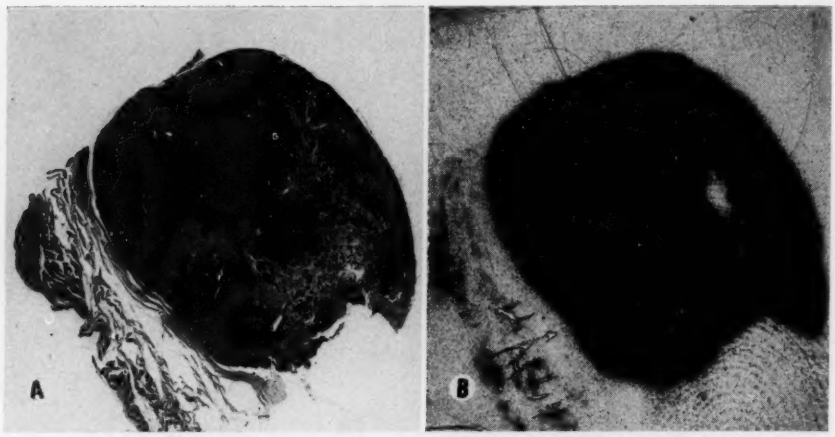

FIG. 4. *H. H. No. 132532.* An unmarried female cook, was born and bred in Belgium. She was well until two years previously, when her fiancé was killed in an accident two weeks before the wedding day. She came to England to work. Thyroid swelling developed three months before coming to the hospital.

Thyroid. There was a soft swelling 3 cm. in diameter in the left lobe. A bruit was heard over it.

Radioactive iodine excretion test:

0– 8 hours,	33.2 per cent
8–24 hours,	5.6 per cent
24–48 hours,	5.9 per cent
Total	44.7 per cent in 48 hours

Neck scanning revealed good radioactive iodine uptake in the nodule.

Operation. Left hemithyroidectomy.

(A) *Pathology.* A large round firm nodule with a solid cut surface and thin capsule, and a smaller nodule with the same general appearance. The larger nodule is seen to contain many areas consisting of large clear cells lying either in solid sheets or forming small active-looking acini. Deep staining, inspissated colloid is present in a minority of these small acini. In contrast to the nodule, the "normal" thyroid tissue appears inactive, consisting of uniform acini with low epithelium and well stained colloid.

(B) *Autoradiograph.* Intense radioactive iodine uptake by the large nodule. None in the rest of the thyroid tissue removed.

radioiodine appears in the colloid of active follicles, inspection of the autoradiographs shows that the nodules in this group are almost completely without function.

Group 5. Multinodular goiter (Fig. 8)

Multiple nodules can be palpated in almost all goiters, both endemic and sporadic, by the time the patient has reached the age of 40. Since it appears that each nodule is primarily a localized area of hyperactivity and

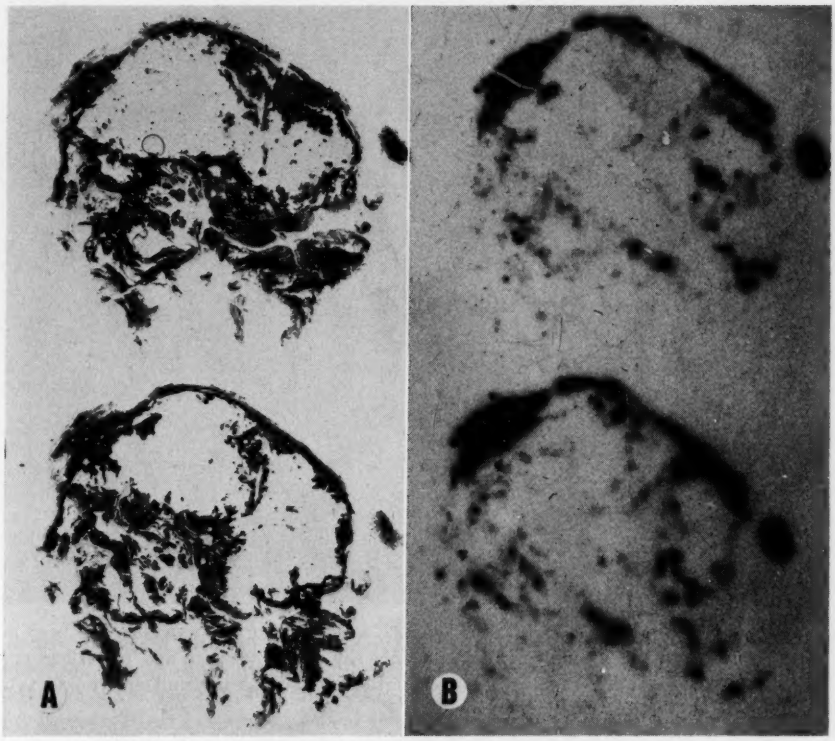

FIG. 5. *H. H. No. 135579.* A housewife, aged 32, was born in a nongoitrous area of Northumberland and had lived in West London for the last fifteen years. Eighteen months before admission, during a third pregnancy, she noticed a swelling the size of a pea in the right side of her neck. During a fourth pregnancy it enlarged and after the birth of the baby, the swelling increased further, doubling its size in six months.

Thyroid. There was a palpable nodule about 4 cm. in diameter in the right lobe.

Radioactive iodine excretion test:

0– 8 hours,	23.5 per cent
8–24 hours,	14.6 per cent
24–48 hours,	0.6 per cent
Total	38.7 per cent in 48 hours

Operation. Right hemithyroidectomy.

(A) *Pathology.* The nodule is composed of large colloid-distended follicles with flattened epithelium. It is surrounded by a few compressed areas of follicles with more active epithelium. The nodule, which has undergone central necrosis, is separated from the rest of the gland by a thin fibrous capsule.

(B) *Autoradiograph.* Intense radioactive iodine uptake is seen in the rind of the nodule, which has undergone central necrosis.

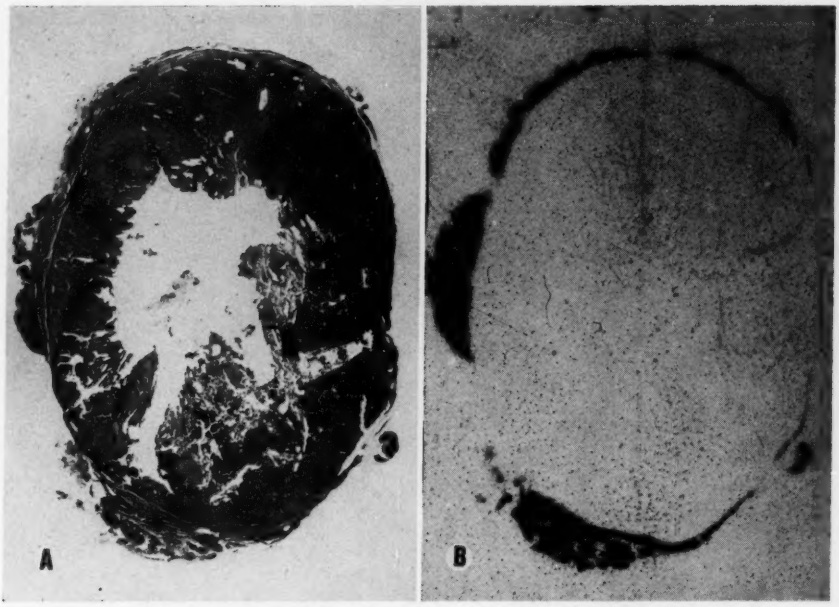

FIG. 6. *H. H. No. 95969.* A spinster, aged 22, a factory worker, was born and bred in West London. She had swelling of the neck since she was 15 years of age, but it had enlarged in the last eighteen months. For six months she had had intermittent choking attacks when lying down.

Thyroid. A firm nodule, about 5 cm. in diameter, was palpable in the isthmus.

Plasma cholesterol level, 176 mg./100 ml.

Radioactive iodine excretion test:

0– 8 hours,	25.4 per cent
8–24 hours,	5.9 per cent
24–48 hours,	1.1 per cent
Total	32.4 per cent in 48 hours

Operation. Removal of nodule with a little adjoining thyroid tissue. (Both thyroid lobes appeared normal.)

(A) *Pathology.* Nodule of thyroid showing mixed macro- and micro-follicular picture. Much of the epithelium is very tall but the colloid is abundant, dense and very little vesiculated. The center of the nodule has broken away.

(B) *Autoradiograph.* No radioactive iodine uptake is seen within the capsule of the nodule. Extranodular tissue shows normal uptake.

later burns itself out, the proportion of inactive to active areas increases with age. Since it also appears that these burnt-out nodules may present a variety of appearances (a group of follicles with flattened cells, a large solitary colloid-filled follicle, a cyst or evidence of old hemorrhage, fibrosis or calcification) it is readily apparent why a multinodular goiter is at first sight so bizarre and pleomorphic.

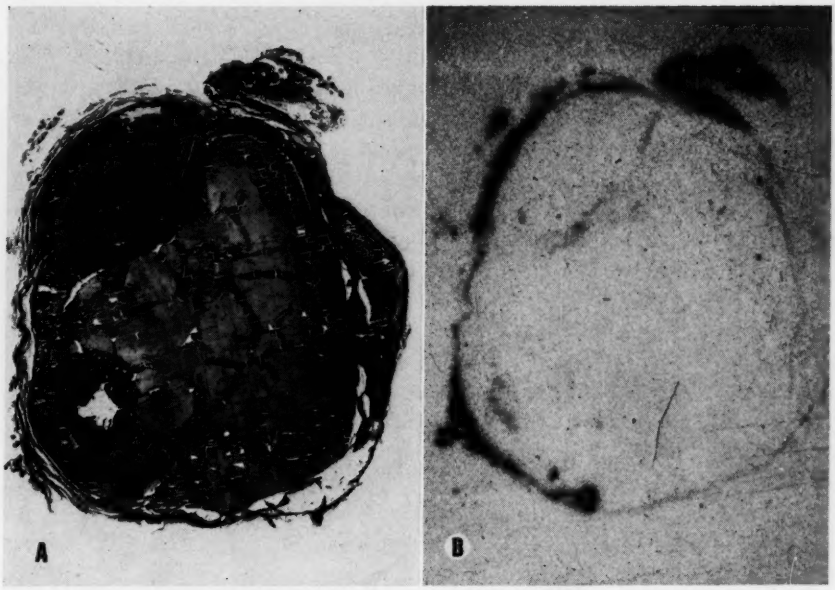

FIG. 7. *H. H. No. 139912.* A female opera singer, aged 32, was born in Italy. She first noticed she could not keep perfect pitch three years before admission; slight swelling of the neck developed. During her first pregnancy eighteen months later, the swelling enlarged but had remained stationary since.

Thyroid. There was a swelling 4 cm. in diameter, replacing the isthmus.

Radioactive iodine excretion test:

0– 8 hours,	47.8 per cent
8–24 hours,	15.8 per cent
24–48 hours,	2.1 per cent
Total	65.7 per cent in 48 hours

Operation. Removal of nodule and a fragment of adjoining tissue. (Remainder of thyroid appeared normal.)

(A) *Pathology.* Apparently a single colloid nodule histologically; except for some central cyst formation, the structure is that of normal thyroid.

(B) *Autoradiograph.* The nodule shows no radioactive iodine uptake, either in its solid part or in the lake of colloid. The extranodular tissue shows normal uptake.

DISCUSSION

The initial change in the thyroid gland in the formation of a goiter is hyperplasia. Such hyperplasia can be due to one of two causes, a low iodine intake, or a goitrogen in the diet, but it is likely that both factors are at work simultaneously in greater or less degree. In areas where goiter is endemic the low iodine intake is the major factor and such goiters are prevented from appearing by giving additional iodide in the diet. Kimball

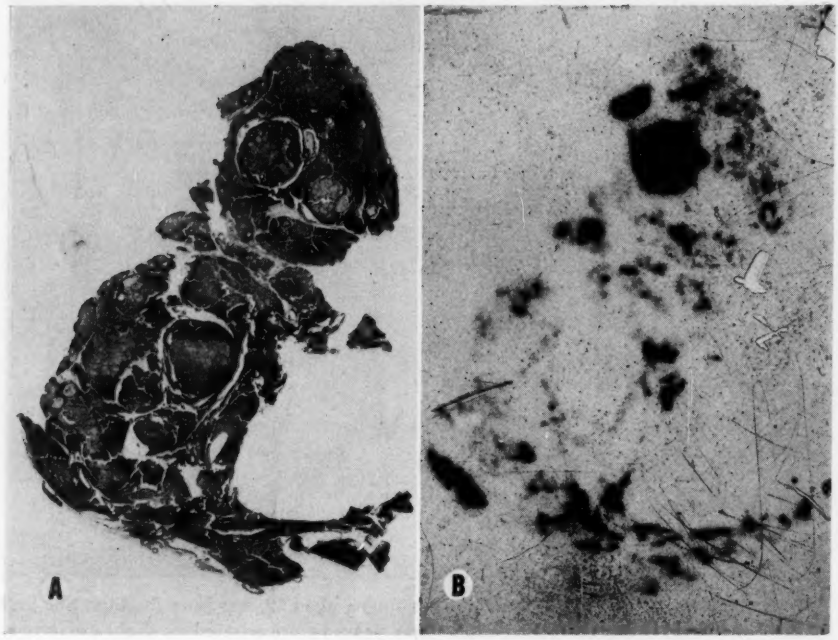

FIG. 8. *H. H. No. 121850.* A housewife, aged 38, was born in a goitrous area of Germany. She underwent thyroidectomy at the age of 15. The swelling recurred in her neck ten years prior to hospital entry and had increased more rapidly in the previous three months. She had had several bouts of choking at night.

Thyroid. Generalized nodular enlargement. Scar of old thyroidectomy.

Radioactive iodine excretion test:

0– 8 hours,	57.1 per cent
8–24 hours,	23.5 per cent
24–48 hours,	6.0 per cent
Total	86.6 per cent in 48 hours

(A) *Pathology.* Multinodular goiter showing a mixture of active and inactive areas, with signs of old hemorrhage and cyst formation.

(B) *Autoradiograph.* The radioactive iodine uptake is confined almost entirely to only one of the numerous nodules.

(9), Marine (10) and others have shown conclusively that in such areas the occurrence of goiter in childhood can be virtually abolished if 100 micrograms of iodide are added to the daily consumption of salt. This does not preclude the possibility that goitrogens are in part responsible for the goiters seen in endemic areas; adequate amounts of iodide can counteract the effects of almost all known goitrogens in the quantities in which they occur naturally in the diet.

Goitrogens. The diffuse hyperplasia and the accompanying autoradiographic evidence of fairly uniform iodine uptake throughout the gland has been seen only in 3 patients, who came from Southern Ireland where goiter is common. From the evidence available (11, 12), the iodine intake is often low in this area, but none of these patients' brothers or sisters showed goiters. In addition there was the possibility of a goitrogen being present in the diet or water supply. All 3 individuals had consumed large amounts of cabbage and turnip twice daily. The only fish eaten was fresh-water trout or pike. The urinary radioactive iodine excretion picture was, however, low and they retained the isotope in a manner similar to the iodide-deficient goiter patients described by Stanbury (13) in Argentina.

In the case of the patient whose thyroid is illustrated in Figure 1, an attempt was made to see if cabbage was a causative factor. He was admitted to the hospital and after three days on a normal diet his urinary radioactive iodine excretion was measured, every two hours for twelve hours, and every twelve hours for thirty-six hours. The test was repeated after he had eaten both raw and cooked cabbage at all meals for four days, but no change was seen in the pattern of his radioiodine excretion. Unfortunately, the test was performed in July, and it is only uncooked winter cabbage which is effectively goitrogenic (McCarrison (14)), although this was not realized at the time. A further test after turnip had been added to all the meals for four days was also without effect.

When the iodide content of the diet is low the presence of a goitrogen may be the factor which actually precipitates endemic goiter. McCarrison's (15, 16) observations on this subject, made fifty years ago, are often overlooked. He studied the incidence of goiter in a group of nine villages which make up Gilgit in the foothills of the Himalayas in Northwest India. In eight of these villages goiter was endemic. They were located one below the other on the same water supply, which became more and more polluted with human and animal excreta as it descended. A goiter survey showed the incidence increased as the water became more polluted. The village with no goiter had a separate supply of spring water. McCarrison filtered off the solid matter from the grossly polluted water and twice daily gave himself and a group of volunteers a drink of 6 ounces of the suspended matter. In one third of the group, including himself, a goiter developed between the tenth and fifteenth days, the enlargement reaching its maximum size between the twenty-fifth and thirtieth days. The goiters all disappeared after the experiment was concluded. The iodine content of the water and diet was low but did not of itself lead to goiter, a fact which was clearly demonstrated by giving a control group of subjects the same polluted water after it had been boiled; in none of them did thyroid swell-

ings develop. Boiling could not have increased the iodine content of the water but must have inactivated or destroyed some goitrogenic substance. A few years later Marine and Lenhart (1909–1910) also studied an epidemic of goiter due to water pollution, but in their case the subjects were trout (17, 18). They found that hyperplastic goiter occurred in artificially reared trout confined in tanks located one above the other on the same water supply, the effluent from one tank flowing into the next below it in the series. The incidence of goiter increased from the first to the last tank.

To recapitulate, the earliest stage of simple goiter is a diffuse hyperplasia with an increased avidity for iodine. It occurs when the iodine content of the diet is low and is probably associated with some goitrogenic substance in that diet. The goiter can be prevented if additional iodine is given and in its early stages this thyroid enlargement is reversible. After the thyroid has remained enlarged for as long as a year the addition of iodine to the diet will no longer bring about a return to normal size.

The second group of goiters showed a completely different picture from the first, both histologically and autoradiographically. The glands showed little or no uptake of iodine, except in a few discrete areas. In these areas activity was great, as shown by intense blackening on the autoradiograph. Histologic examination showed that they were composed of groups of follicles of uniformly small diameter with tall cells and prominent nuclei (6, 7).

In a previous study of these active areas from 6 nontoxic goiters the diameters of 100 follicles were measured and compared with the diameters of 100 follicles situated in a nonactive part of the same gland. Table 1 shows the measurements obtained, and it is seen that the average diameter of these follicles was less than one third that of the inactive ones. The second striking feature of the histologic picture in these areas, corresponding to the blackening on the autoradiographs, was that the diameters of these active follicles were extremely uniform as compared to the wide range of diameters seen in the inactive follicles. This is well shown in Figure 9. The findings could conveniently be described as "toxic follicles in non-toxic goiter."

These areas were more vascular than the remainder of the gland and in some, condensations of tissue developed at the periphery, thus becoming nodules. It is characteristic of the thyroid follicle to pass through alternating phases of activity and rest (19, 20) but in these glands it would appear that all the follicles which were active at any one time were grouped together. Possibly only those follicles of a certain critical size were targets for the thyroid-stimulating hormone of the anterior pituitary. The reason for their being grouped together may be due to the arrangement of blood vessels. However, their great activity would necessitate increased vascularity and it is not easy to see which comes first, activity or vascularity.

TABLE 1. AVERAGE DIAMETERS OF FOLLICLES IN 6 NONTOXIC GOITERS

(One hundred follicles measured in two diameters at right angles to each other, in both active and nonactive areas of each goiter)

Case No.	Average diameter in microns	
	Active follicles	Inactive follicles
1	63.2	204.8
2	88.8	201.0
3	86.2	161.7
4	83.1	204.8
5	50.5	140.6
6	50.0	177.4

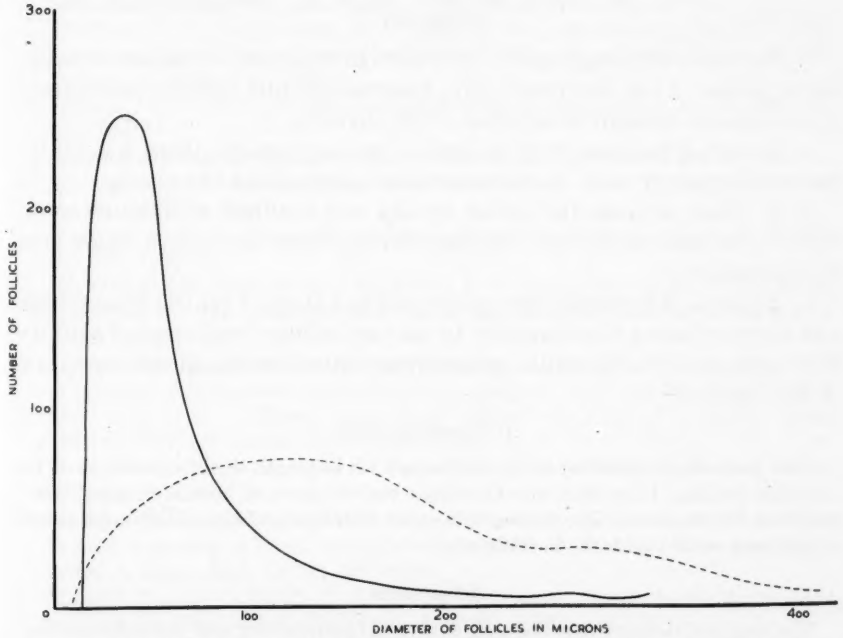

FIG. 9. Distribution of follicles in active and inactive areas of thyroid in 6 patients with nodular goiter. Graph shows distribution of size of follicles in active (continuous line) and in inactive (interrupted line) areas. Note uniform diameters of active follicles and widely scattered diameters of inactive ones.

The rare finding of a solitary hyperactive nodule in the thyroid is of particular interest. The one shown in Figure 4 occurred in a girl in whom the goiter developed two years after her fiancé was killed in an accident,

just before they were to be married. There was no evidence of hyperthyroidism. It is possible that such a patient might become frankly hyperthyroid but the disease appeared to be self-limiting, since hemorrhage had already started to destroy the center of the nodule.

All the nodules appeared to be subject eventually to central hemorrhage and necrosis, a fact well shown in Figure 5. Thyroidectomy was performed soon after necrosis had taken place, as shown by the remaining rind of active tissue. This phenomenon was described by Dobyns and Lennon (8). Combined with the fact that new groups of follicles can enter a phase of hyperactivity, this process leads to the production of more and more nodules. The inactive or burnt-out nodules show a variety of histologic pictures, *e.g.*, a lake of colloid with or without groups of follicular cells, an organizing blood clot, fibrosis, or calcification.

SUMMARY

1. Patients with simple goiter have been investigated by means of radioactive iodine. After thyroidectomy, autoradiographs were prepared from sections made through whole lobes of the thyroid.

2. In young patients from an area of endemic goiter, there was an increase in iodine uptake, which was uniform throughout the gland.

3. In older patients the iodine uptake was confined to discrete areas, made up of uniformly small follicles. Rarely, there was only a single area of hyperactivity.

4. All areas of hyperactivity progressed to a stage of central hemorrhage and necrosis, losing their capacity to take up iodine. Fresh areas of activity then appeared. Multinodular goiters represented the continued repetition of this process.

Acknowledgments

I am particularly indebted to Dr. I. Doniach for help with the interpretation of the histologic findings. I also wish to acknowledge the assistance of Miss E. Poulson, B.Sc., and Miss M. Tomlinson. The photographs of the histologic and autoradiographic preparations were made by Mr. E. V. Willmott.

Addendum

The results of radioactive iodine tests of thyroid function vary with the technique used and the district in which the tests are performed. The following are the limits of the normal range for the results of the urinary excretion test as carried out in this hospital:

0– 8 hours	25–50 per cent
8–24 hours	7–20 per cent
24–48 hours	0–10 per cent
Total	35–70 per cent

The test is usually carried out, using a tracer dose of 5 to 10 microcuries of radioactive iodine, I^{131}.

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SEXUAL PRECOCITY IN A 5-YEAR-OLD BOY WITH INTERSTITIAL-CELL TUMOR OF THE TESTIS

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THERE have been previously recorded only 9 cases of sexual precocity due to the hormonal effects of an interstitial-cell tumor of the testis. The pertinent literature has been reviewed by Cook *et al.* (1).

The following case is reported in order to aid in the characterization of the clinical and endocrinologic features of this unusual syndrome.

CASE REPORT

N. R., a white male child, was first seen at the age of 5 years on September 4, 1950. He had been born on June 7, 1945. He was the product of a first pregnancy and weighed 6 pounds 15 ounces, and measured 21½ inches in length at birth. Early pediatric development was not remarkable. In 1949 during his fourth year, his general body growth was noted to be rapid and in January 1950 the mother noted a few pubic hairs developing. Since that time until September 1950 when he was first admitted to our clinic, there was progressive and rapid genital development, frequent acneform eruption about the face and shoulders, and a marked change in his voice. His health had otherwise been good, except for recurrent tonsillitis necessitating a tonsillectomy in May 1950.

Past illnesses included roseola infantum at 16 months and Vincent's infection of the mouth at 18 months.

Social history indicated good general adjustment in home and family relationships and in play patterns with other children.

One brother, who is 2½ years old at the present time, has shown no abnormalities to date. The mother's half-brother is known to have had very early sexual development. He has been married for twelve years and is childless.

Physical examination at this admission revealed a well developed 5-year-old boy, whose height was 47½ inches and weight 50 pounds (Fig. 1). Somatic proportions were normal. The boy walked with a list to the left and his spine showed a compensating scoliosis. Evidence of previous acne was present on the face and trunk and there were several scattered pigmented naevi on the trunk but there was no abnormal pigmentation. The skin was well sun-tanned. Hair on the head was normal in amount and texture. Axillary

Received for publication March 7, 1953.

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and facial hair was absent and there was only slight development of body hair. Pubic hair was well developed (Figs. 1 and 2). Heart and lungs were normal. Blood pressure was 90/60. Neurologic examination showed the cranial nerves to be grossly normal. Dental examination revealed that only the upper central incisors had been replaced. There were no palpable enlarged lymph nodes. The penis was circumcised and showed development corresponding to that in a 14-year-old boy. The scrotum contained enlarged testes and there was good development of the sac and rugae. There was no hypospadias or other penile abnormality. Rectal digital examination revealed prostatic development similar to that of early adolescence. The musculature and extremities were not remarkable. His voice had a nasal quality and lacked normal soprano tone. The patient was highly intelligent; his speech and motor coordination were excellent.

Neurologic study, including an electroencephalogram, revealed no abnormality. Visual fields were normal and vision was 20/22 O.D. and 20/40 O.S.

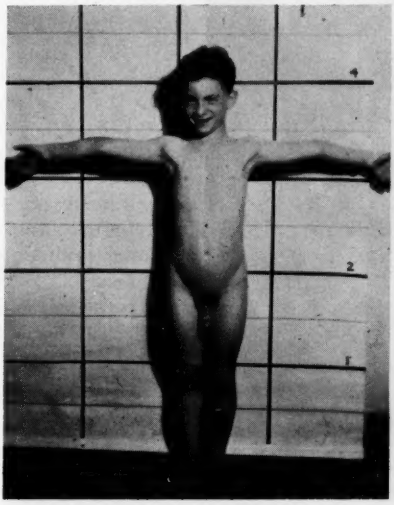

FIG. 1. N.R., September 4, 1950—aged 5 years.

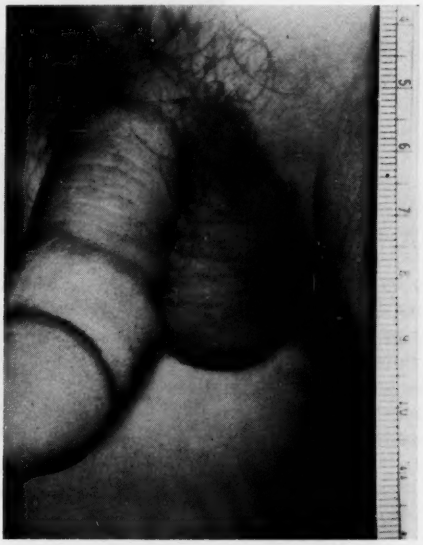

FIG. 2. Close-up of genitalia, showing enlarged left testis.

A pyelogram was unsatisfactory; x-ray examination showed the skull to be normal, and the bone age was estimated to be about 10 years, because of the full development of the pisiform bone.

The drop in the eosinophil count following a glucose tolerance test was from 144 cells /cu. mm. to 48 cells/cu. mm. The results of a glucose tolerance test, hemogram, and urinalysis were normal.

The patient showed steady progress in sexual development. This was recorded by periodic photographs. He grew 3½ inches in eight months. Meanwhile, the left testis was noted to undergo a disproportionate enlargement, and a minimal alteration in consistency to palpation suggested the presence of tumor. Exploration on May 19 ,1951, when

he was almost 6 years old, revealed a brownish nodular tumor in the parenchyma of the left testis (Fig. 3). *The left testis was removed.*

The patient's *course since operation* has been entirely uneventful. On May 15, 1952 (one year after his left orchiectomy) he was the size of an average 9 or 10-year-old boy; his height was 53¾ inches and he weighed 68 pounds. His penis remained large and measured 9 cm. in length, and his remaining testis was 5 cm. ×2 cm. Although initial measurements of this testis were not recorded, photographic comparison indicated that it had undergone slight enlargement. His prostate had atrophied markedly and his acne had not recurred. Although he had frequent erections, no emissions or masturbation were

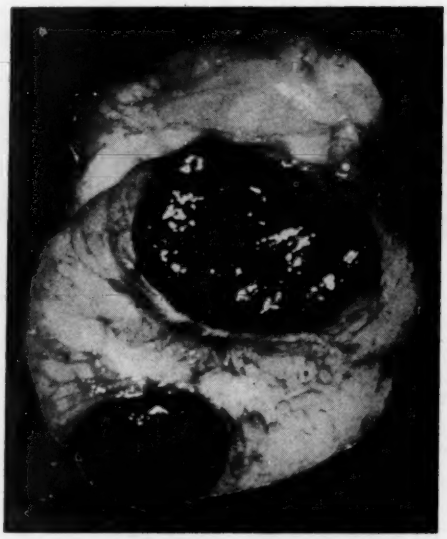

FIG. 3. Cut surface of left testis, showing position and size of tumor.

noted. His voice remained harsh and coarse; pubic hair had not regressed, and it appeared that the status of his secondary sex characters was stationary. By October 20, 1952, seventeen months after operation, he was noted to be 55½ inches tall and to weigh 73 pounds. X-ray examination of the wrist bones showed no undue skeletal progress and his sexual development again seemed stationary. However, no evidence of regression was apparent, except for further atrophy of the prostate gland.

The course of urinary *17-ketosteroid excretion* was followed, employing a modification of the method of Holtorff and Koch (2) (Table 1).

Further tests of eosinophil response and carbohydrate tolerance after operation agreed with the preoperative normal values.

Pathologic study of the surgical specimen (Fig. 3) revealed a sharply circumscribed, but encapsulated, brownish tumor mass with a small amount of compressed collagenous stroma about the periphery. The surrounding testis was composed of seminiferous tubules lying in a loose edematous stroma with little or no interstitial tissue, except for a

TABLE 1. URINARY 17-KETOSTEROIDS IN RELATION TO LEFT ORCHIECTOMY

Date	Urine vol. (cc.)	17-KS (mg./24 hrs.)
	BEFORE OPERATION	
Mar. 15, 1950	550	3.3
June 13, 1950	280	2.0
Sept. 6, 1950	550	4.0
May 10, 1951	480	2.6
May 11, 1951	380	2.1
May 19, 1951	*Left orchiectomy*	
	AFTER OPERATION	
Jan. 26, 1952	280	0.9
May 3, 1952		2.6
Nov. 1, 1952	536	2.3
Nov. 2, 1952	750	2.6

few connective tissue cells and small blood vessels. No Leydig cells were seen. For a 5-year-old boy, the seminiferous tubules showed an unusual degree of proliferation, and maturation to secondary spermatocytes in some tubules.

The tumor (Fig. 4, A and B) was composed of large polygonal cells with round or slightly oval nuclei which were rather hyperchromatic and frequently contained a single large nucleolus. The cytoplasm was abundant, eosinophilic, and granular, tending to become foamy at the periphery of the cell. Many of the cells contained yellow-brown granular lipofuscin pigment, which stained with a periodic-acid Schiff technique. The cell membranes were rather well demarcated and there was an abundant fine reticular stroma which appeared to surround each cell. Fat stains revealed finely granular lipoid scattered in many of the cells of the tumor.

DISCUSSION

This case agrees in many of its features with the generalization drawn by Cook *et al.* (1).

The clinical manifestation of the disorder in the present instance corresponds to that in the 5 to 6-year-old groups described by them and by the earlier authors whose observations they summarize. The relatively slow but steady progress of sexual and somatic development is characteristic. However, our patient showed little alteration in musculature. The degree of regression of the precociously developed secondary sex characters after surgical excision of the tumor is reported to be highly variable. In the present case, regression was noted with respect to the acne and the size of the prostate gland. The enlargement of the penis and the pubic hair have persisted, but there has been no apparent progression in any of these second-

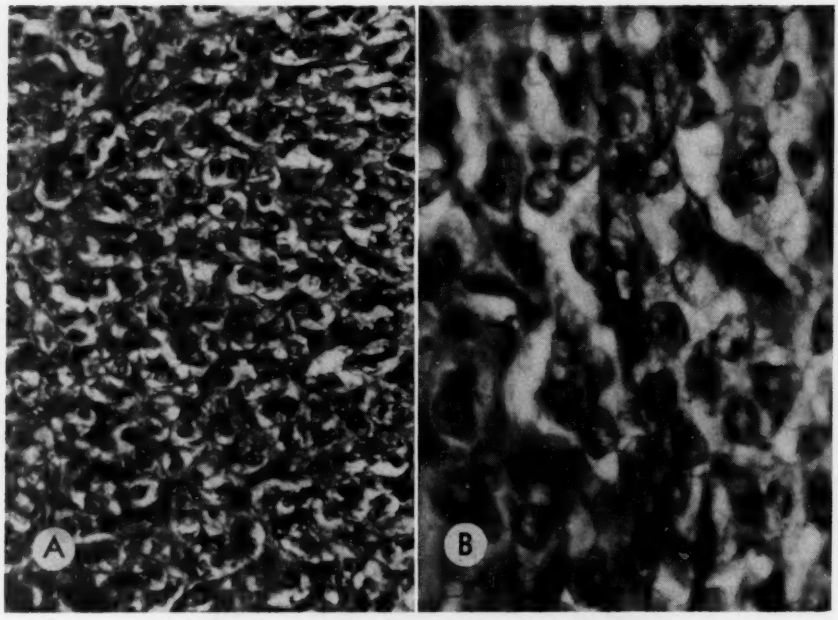

FIG. 4. A. Representative area of tumor (periodic-acid Schiff stain) ×300.
B. Representative area of tumor (periodic-acid Schiff stain) ×935.

ary sex characters and the voice has remained essentially the same as before surgery.

The possibility remains that either in the remaining testis, the adrenal glands, or elsewhere in the body, an additional nodule of androgen-producing tissue persists or has developed following the left orchiectomy. The postoperative urinary 17-ketosteroid determinations suggest this possibility and the boy is being closely followed.

Unlike the case of Cook *et al.*, this boy presented no evidence of deviation from normal carbohydrate and mineral metabolism. Moreover, he was initially normotensive and has remained so during our entire period of observation.

Functioning tumors associated with precocious pubertal masculinization arise in either of the two sites capable of androgen production, the testis or the adrenal gland. Morphologically these tumors are similar and often indistinguishable from one another. Grossly they are usually well circumscribed, fairly soft, and often well vascularized, and yellow or brown. Microscopically they consist of polygonal cells with round or oval nuclei and fairly abundant eosinophilic cytoplasm, which often contains small

lipoid droplets and occasionally some fine brown lipofuscin pigment. At times these cells are each surrounded by a delicate network of reticulum. This cell type and pattern is seen in tumors either of the adrenal cortex or of Leydig (interstitial) cells of the testis. As one might expect, the location of the tumor usually indicates its origin. If the tumor is in the adrenal gland it is adrenocortical in type; if it is in the testis it is usually of the interstitial-cell type. However, adrenocortical rests are common outside the adrenal gland and particularly along the spermatic cord and in the epididymis. This is apparently due to the close anatomic approximation of these structures during embryonic development from the urogenital ridge. For this reason, tumors of adrenocortical origin may develop in the testis or more commonly in the epididymis. The only morphologic characteristic which will distinguish between adrenocortical cells and interstitial cells of the testis is the occasional presence of crystalloids of Reinke. These crystalloids are found only in interstitial (Leydig) cells and are not characteristic of adrenocortical cells. In the case described here, no crystalloids were observed. Although it is conceivable that cells of adrenocortical origin may produce merely androgenic hormones, it is apparent from most studies of functional adrenocortical tumors that other adrenocortical hormones are also produced.

In the case of Cook *et al.* (1) there was, in addition to evidence of androgenic activity, eosinopenia, a retention of sodium and chloride, a slightly elevated blood sugar and a slight increase in blood pressure. These physiologic findings would suggest an adrenocortical rather than an interstitial-cell tumor, especially since the values returned to normal after removal of the tumor. In contrast, our case showed none of these characteristics and would appear more properly to be regarded either as a rare adrenocortical tumor producing only androgenic hormone or, more likely, a tumor of the interstitial cells of the testis.

SUMMARY

The clinical and laboratory findings in the case of a 5-year-old boy with sexual precocity due to interstitial-cell tumor of the testis are presented. These data, viewed in relationship to previously recorded observations in the literature on 9 cases, may aid in the characterization of this unusual syndrome.

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AN UNUSUAL OVARIAN TUMOR CONTAINING LEYDIG CELLS BUT ASSOCIATED WITH ENDOMETRIAL HYPERPLASIA, IN A POSTMENOPAUSAL WOMAN

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OVARIAN tumors containing cellular elements characteristic of the male gonad have been generally considered to fall into two categories from an endocrinologic viewpoint: a) androgenic, and b) nonfunctional. Only recently have the estrogenic potentialities of these tumors been suggested (1). The following case is one of an ovarian tumor which appeared to be estrogenic in spite of its content of large numbers of Leydig cells.

CASE REPORT

Clinical history. **A. O'D. (M.G.H. #13086)**, a 64-year-old widow, mother of one child who died in infancy, was admitted to the hospital because of bloody vaginal discharge of several days' duration. Her menopause had occurred twenty years before. There had been no subsequent bleeding until two years prior to entry, when an episode similar to the presenting one had taken place. The past history had been otherwise unremarkable. The findings on physical examination were essentially negative, except for an enlarged uterus. Following the examination bright blood emanated from the cervical os. The blood pressure was 150 systolic and 80 diastolic. The red blood count was 4,330,000. A dilatation and curettage specimen revealed cystic hyperplasia of the endometrium and a small focus of adenocarcinoma (Figs. 1 and 2).

Two weeks later a total hysterectomy and bilateral salpingo-oophorectomy was performed.

Recovery was uneventful. At a follow-up visit six months postoperatively the patient stated that she was "sure" she was having hot flashes. She continued to complain of these for over a year longer. She was last seen eight years and nine months postoperatively, at which time there was no evidence of tumor recurrence.

Pathology. Curettings (37-1534). (One section, hematoxylin and eosin.) Eight fragments ranging from 0.1 to 1.2 cm. in diameter were endometrial in origin. The glands varied considerably in size and shape; many were cystic (Fig. 1); others showed varying degrees of papillary infolding. In several areas the glands were "back to back." The papillary infolding and the approximation of the glands in one small fragment were sufficiently marked to warrant a diagnosis of focal early carcinoma (Fig. 2). Under high

Received for publication January 12, 1953.

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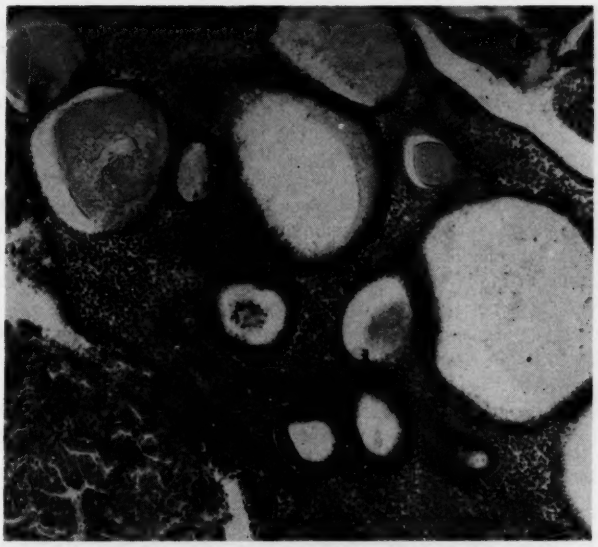

FIG. 1. Endometrial curettings, showing cystic hyperplasia. Note marked variation in size of glands. A small portion of the fragment shown in Figure 2 is also seen here. ×80.

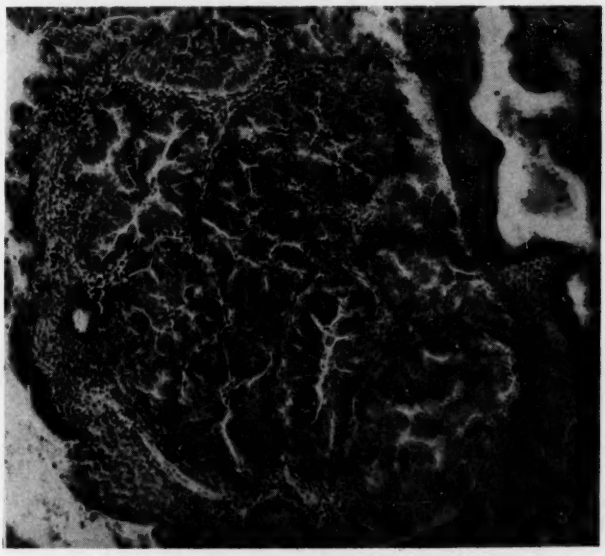

FIG. 2. Endometrial curettings, showing focus of early adenocarcinoma. Note papillary infolding and "back-to-back" position of glands. A small portion of the fragment of cystic hyperplasia shown in Figure 1 can be seen along one margin. ×80.

power, the glands were seen to be lined by pseudostratified columnar epithelium, which showed considerable mitotic activity. The stroma was composed predominantly of plump cells of the "naked nucleus" type which characterize active proliferative endometrium; occasional stromal mitoses were encountered. Several endometrial sinusoids were thrombosed, and there were a few foci of endometrial necrosis.

Uterus, tubes, and ovaries (37-1818). The uterus measured 10.0×7.0×4.0 cm. The myometrium ranged from 1.0 to 3.0 cm. in thickness and contained several leiomyomas, the largest of which was 4.0 cm. in diameter. The endometrium was soft and measured less than 0.1 cm. in thickness; in the region of the internal os it was irregularly granular and slightly hemorrhagic. The cervix and tubes were unremarkable. In two hematoxylin and eosin sections of the lower uterine segment and endocervix the findings were essentially negative.

The right ovary measured 2.0×2.0×0.5 cm. The left ovary was completely replaced by a 3.0×2.5×2.0-cm. firm, nodular, yellowish-pink mass; its sectioned surface was bright orange-yellow.

Twelve hematoxylin and eosin sections from seven blocks of the tumor and one Sudan IV section from an additional block were available for microscopic study. Examination

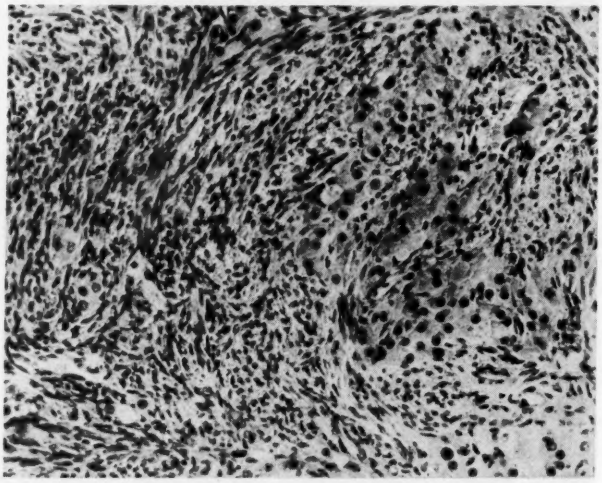

FIG. 3. Ovarian tumor. Spindle cells are arranged in intersecting fascicles. Nests of polyhedral cells with abundant dense cytoplasm and round nuclei are visible at right. ×200.

under low-power magnification revealed intersecting bundles of spindle cells separated by collagen fibers (Fig. 3). Here and there were bands and patches of hyalinized collagen; some of these had sharply etched borders but in others the borders were ill-defined. Disseminated throughout the tumor were numerous large polyhedral cells with dense eosinophilic cytoplasm (Fig. 3). These cells occurred individually as well as in islands of up to 200 (in a single plane of section). In one area within the tumor there were several small rounded nests and cords of cells of epithelial type (Fig. 4). Groups of large polyhedral cells nestled against these cells, here and there. Under higher powers (Figs. 5 and 6) the spindle nuclei were uniform and contained evenly distributed, fine chromatin gran-

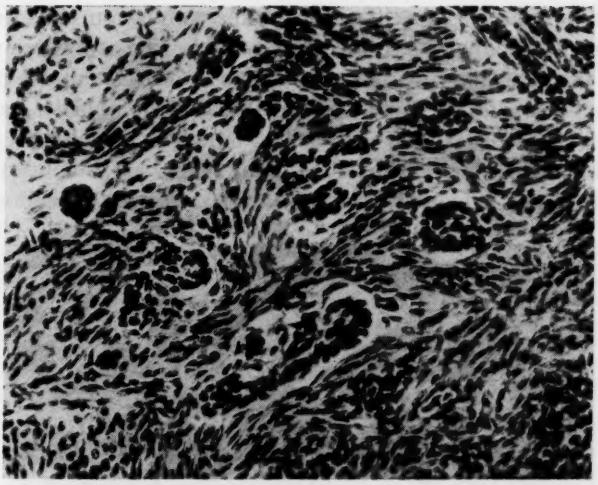

FIG. 4. Ovarian tumor. Small nests of cells of epithelial type are seen surrounded by the spindle cell element of the tumor. ×250.

ules; no mitoses were encountered. The large polyhedral cells had uniform round nuclei which often contained single or a few nucleoli; in the cytoplasm of many of these cells were one or more large eosinophilic rods (Figs. 5 and 6)—typical crystalloids of Reinke. The smaller epithelial cells had no discernible cytoplasm; their nuclei were oval to elongated, and often were grooved. Sudan IV stain showed small droplets of fat in the polyhedral cells and to a lesser extent in some of the spindle cells.

One section of the right ovary showed an abundance of stroma in the outer cortex. This was characterized by fascicles and whorls of thin, wavy collagen fibers; the stromal nuclei were small and pale and ranged in shape from oval to elongated. Within the stroma were a few nests of polyhedral cells with abundant eosinophilic cytoplasm (so-called luteinized stromal cells), and rare clusters of lymphocytes and epithelioid cells (so-called cortical granulomas) (2). Numerous corpora albicantia and atretica were present in the central portion of the ovary.

COMMENT

The evidence is in favor of estrogen production by an ovarian tumor composed of testicular cell types, for the following reasons:

1. The patient presented a typical clinico-pathologic syndrome of hyperestrinism—postmenopausal bleeding from an actively hyperplastic endometrium.[1] The identification of the hormone or hormones producing the syndrome is necessarily speculative, as hormone assays were not performed. Although it is possible that the hyperplastic endometrium was an

[1] Endometrial carcinoma, of which there was a small focus, may be regarded as an additional manifestation of hyperestrinism; endometrial carcinoma is frequently associated with estrogen-producing ovarian tumors (3).

unusual end-organ response to androgenic stimulation, this is unlikely. The endometrial changes described in human females to whom androgens have been administered have varied from none (with small doses or during the secretory phase of the cycle) to hypoplasia, atrophy (4) or basal vacuolization of the glands (postmenopausal endometria primed with estrogens) (5). Endometrial hyperplasia, to our knowledge, has not been reported. At the very least, it is admissible to say that in this patient one of the

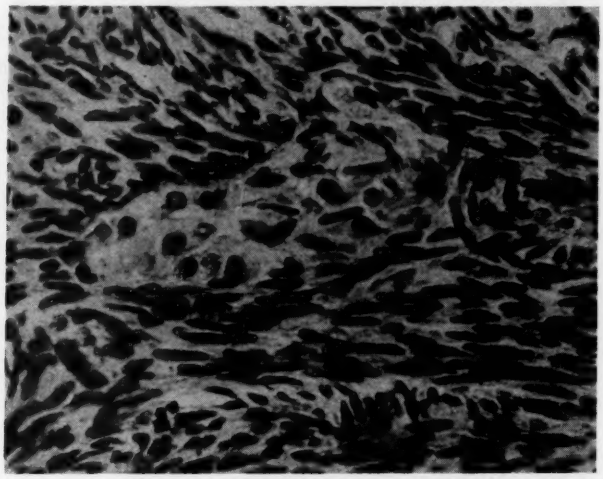

FIG. 5. Ovarian tumor, showing nest of Leydig cells surrounded by spindle cells. In the right portion of the Leydig cell nest two dense rod-shaped crystalloids of Reinke may be clearly seen. ×350.

typical biologic pictures of hyperestrinism in human females was duplicated; in this sense, an estrogenic influence was at work.

2. The experience of hot flashes after operative intervention suggests that the source of the hormonal stimulation must have been some component of the operative specimen.

3. The two conceivable sources of hormone in the operative specimen were the non-neoplastic ovary and the tumor. The former did contain an excess of cortical stroma, in which were a few nests of luteinized cells. However, the spindle cells of the stroma had small inactive-appearing nuclei, and the luteinized cells, though they were morphologically consistent with steroid secretion, were quantitatively very few in number. Much more likely as a source of hormone was the tumor, which contained spindle cells with nuclei considerably larger than those of the cortical stroma, and numerous large polyhedral cells that had an appearance often seen in steroid-secreting tumor cells.

4. The tumor, from a morphologic viewpoint, was capable of three interpretations: granulosa-theca cell tumor, tumor of mixed female and male elements (gynandroblastoma (6)), and Sertoli-Leydig cell tumor (arrhenoblastoma-androblastoma series of Teilum (1)). That the tumor was of pure granulosa-theca cell nature can be dismissed, since at least some of its component cells were unquestionable Leydig cells. Their positive identification was based on the presence in their cytoplasm of the highly specific

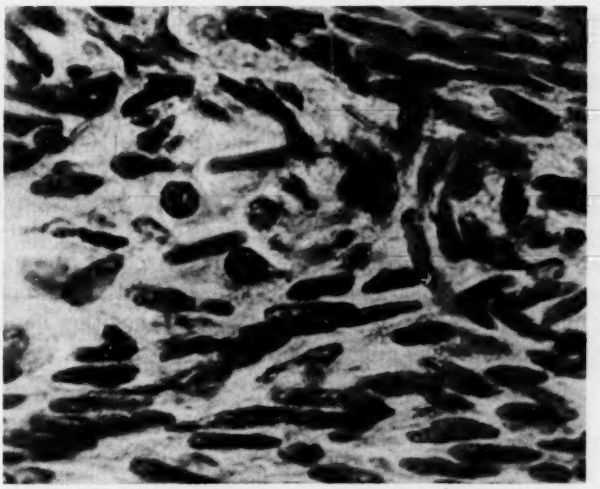

FIG. 6. Ovarian tumor. High-power view of area in Figure 5 containing crystalloids. Latter are seen just to left of center. ×1500.

crystalloids of Reinke (Fig. 6). These structures have been encountered only in the Leydig cells of the testis, and of the embryonic testicular remnant in the ovarian hilus. (In the latter site the Leydig cells are commonly referred to as hilus cells.) On the other hand, crystalloids have never been seen in granulosa-lutein cells, theca-lutein cells, or luteinized cells of the ovarian stroma—all cells otherwise similar in morphology to Leydig cells. The diagnosis of tumor of mixed female and male cellular elements (gynandroblastoma) was suggested by the co-existence of Leydig cells and of spindle cells growing in a thecoma-like pattern. It could be argued that at least some of the spindle cells and large polyhedral cells were theca cells, derivatives of female mesenchyme, and that these cells represented the estrogenic element of the tumor. The evidence against this was that ovarian tumors of granulosa-theca and Sertoli-Leydig cell types recapitulate embryonic organization, and rarely, if ever, contain proven cellular elements of both female and male types. That a homogeneous, differentiated

tumor such as the one under discussion should be composed of an intimate mixture of mature female and male cells seems unlikely in our opinion. Finally, we favored the interpretation of this tumor as a pure Sertoli-Leydig cell neoplasm. All the polyhedral cells were morphologically compatible with Leydig cells; the absence of crystalloids in some of them did not exclude their Leydig-cell nature. The spindle cells, intimately associated with the polyhedral cells, could be interpreted as derivatives of male mesenchyme (spindle cells in fascicular pattern are encountered in virilizing arrhenoblastomas as well as in thecomas). The small epithelial-type cells were nonspecific in structure so far as their female or male origin was concerned; taken alone, they could be considered either granulosa or Sertoli cells. However, in this tumor, their close association in growth with the Leydig cells suggested strongly that they were Sertoli cells.

If this was a functioning tumor composed entirely of male elements, the hormone-producing cells appeared to be the Leydig cells and, possibly, the intimately related spindle cells. Both types of cells contained fat droplets; the Leydig cells, in addition, were characterized by abundant eosinophilic cytoplasm, an appearance consistent with active steroid secretion. The cells which appeared to be Sertoli in type, on the other hand, were few in number and had an insignificant content of cytoplasm.

DISCUSSION

Strong evidence exists that the male gonad is capable of estrogen production (7). Substances having estrogenic effects have been recovered from stallion, bull, and human testes; and crystalline alpha-estradiol and estrone have been isolated from stallion testes. Feminizing tumors arising from testicular elements are not uncommon in the dog (8). Finally, a number of cases of male pseudohermaphrodism, characterized by an association of female secondary sexual development and either cryptorchid or descended testes have been reported (9). Such a case may show normal estrogen values, which are reduced to the castrate level by removal of the testes (10).

Although it is universally agreed that androgens are formed by the Leydig cells of the testis, the site of estrogen production is controversial. The canine testicular tumors just mentioned, from which estrogenic hormones can be recovered in large quantities (11), are tubular tumors of Sertoli-cell origin, and constitute strong evidence that, at least in dogs, these cells are capable of estrogen production (12). The evidence for Sertoli-cell estrogen production in man is weaker, but still suggestive. Teilum (1) has reported a Sertoli-cell tumor in a human testis, similar morphologically to the canine tumor; it was associated with increased urinary estrogens, and clinical effects attributable to hyperestrinism (gynecomastia, loss of

libido, and impotence). Other endocrine abnormalities of the human testis, and animal experimentation afford confirmatory evidence that the Sertoli cells are the source of an estrogen-like hormone (7).

There is likewise evidence that the Leydig cell is capable of producing an estrogenic hormone. A case has been reported (13) in which postmenopausal bleeding and painful swelling of the breasts with secretion were associated with marked Leydig (hilus) cell hyperplasia of the ovaries.[2] Bilateral oophorectomy and hysterectomy was followed by regression of symptoms and the onset of hot flashes. Husslein (14) has reported 4 cases of postmenopausal cystic hyperplasia of the endometrium that were associated with hyperplasia or small tumors of the ovarian hilus cells. Finally, Maddock and Nelson (15) have shown that administration of chorionic gonadotropin to the human male causes an increase in urinary estrogen output, the histologic counterpart of which is a hyperplasia of the Leydig cells of the testis; the Sertoli cells do not show hyperplasia in such cases. Moreover, this increase in estrogen output can occur in an individual with Addison's disease (suggesting that the adrenal cortex does not play an important role in the estrogen production), but does not occur in a castrate.

Despite the evidence that testicular elements are capable of estrogen production, pathologists in diagnosing functional ovarian tumors have been loathe to consider the possibility that tumors composed of testicular cell-types may be estrogenic; the tendency has been to regard all estrogenic ovarian tumors as granulosa-theca cell tumors, regardless of morphology.

Teilum (1) has recently pointed out that so-called tubular granulosa-cell tumors—tumors associated clinically with manifestations suggestive of estrogen secretion—may be histologically indistinguishable from the tubular adenoma variety of arrhenoblastoma. He regards such tumors as estrogenic Sertoli-cell tumors (androblastoma tubulare lipoides) rather than granulosa-cell tumors. He has likewise demonstrated the histologic resemblance of "folliculomes lipidiques"—rare lipid-rich ovarian tumors that may be accompanied by endometrial hyperplasia—to Sertoli-cell tumors of the canine testis and to the human testicular Sertoli-cell tumor which he reported. The case described here is one of an unusual ovarian tumor, which appeared to be causally related to the presence of cystic hyperplasia of the endometrium in a postmenopausal woman but which, unexpectedly, contained large numbers of Leydig cells when examined under the microscope.

Besides the evidence that male cell types may be estrogenic, experimental and clinical evidence likewise exists that the ovarian theca cells are potentially androgenic (16). It is therefore apparent that determination of

[2] Luteinized stromal cells of the ovarian cortex were also recognizable in the published photomicrographs, although they were not mentioned as such in the text.

the origin and nature of functioning ovarian tumors cannot always depend solely on their associated clinical and endocrinologic pictures. Rather, the cytologic features of such tumors should be investigated with great care and interpreted, as far as possible, independently of the endocrinologic aspects of the case.

SUMMARY

An ovarian tumor associated with endometrial hyperplasia in a postmenopausal woman, but containing large numbers of Leydig cells, is reported.

Evidence is presented from the literature that testicular elements in normal, hyperplastic, and neoplastic states may produce estrogens.

In view of the feminizing potentialities of testicular cell types, plus the evidence that ovarian cells may be capable of producing androgens, the hormonal aspects of functioning ovarian tumors cannot be used as the sole criteria for their identification. Rather, a careful evaluation of the histology of these tumors is also required for determination of their origin and nature.

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IDIOPATHIC HYPOPARATHYROIDISM AND CHRONIC ADRENAL INSUFFICIENCY: A CASE REPORT

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THE clinical syndrome of idiopathic hypoparathyroidism has recently been reviewed by Steinberg and Waldron (1). They accept the criteria previously established by Drake and associates (2)—low serum calcium level, high serum phosphate level, normal renal function, normal bones by x-ray examination, and chronic tetany—but make several minor modifications. These include the requirement that the serum phosphorus level be above 5 mg. per 100 ml. in adults and above 7 mg. per 100 ml. in persons under the age of 16, and that the clinical course be not characterized by chronic diarrhea.

The coincidence of idiopathic hypoparathyroidism and chronic adrenal insufficiency (Addison's disease) has been reported in 3 previous cases to our knowledge. Only 1 of these, however, (that reported by Leonard (3) in 1946) would seem to have been sufficiently documented to make both diagnoses certain. The foregoing criteria for the diagnosis of idiopathic hypoparathyroidism were met in this case, and autopsy revealed complete fibrosis of the adrenal cortices ("primary atrophy") and total absence of the parathyroid glands. The 2 other cases which probably represented combined parathyroid and adrenal insufficiency, cannot be accepted unequivocally. The first (Case #3 of Sutphin, Albright, and McCune (4)) was a 12-year-old boy with hypocalcemia and tetany, who subsequently showed pigmentation, hypotension, and weight loss which terminated fatally; but there was no documented hyperphosphatemia (above 5 mg. per 100 ml.), adrenal function studies could not be made, and there was no autopsy. The second, (an 8-year-old girl reported by Talbot, Butler and MacLachlan (5)) had well demonstrated hypoadrenalism as well as hypoparathyroidism, but the details of the latter were not reported and, therefore, cannot be evaluated.

The patient in this report meets all the described criteria, except that his course has been modified by intermittent diarrhea. We believe that the diagnosis of idiopathic hypoparathyroidism is, nonetheless, adequately

Received for publication April 10, 1953.

established, and the case is presented as an example of what we believe to be well documented Addison's disease in addition.

P. H. (Pres. Hosp. Unit #98-01-58) was a 20-year-old male, who had been followed in in this Hospital with idiopathic hypoparathyroidism for ten years and who had recently shown the additional signs of adrenal cortical hypofunction. He was first admitted in 1942, at the age of 10, because of diarrhea and convulsions. His birth and early development had apparently been normal. Parents and an only brother were normal, except that all gave a history of childhood eczema. The onset of asthma occurred at the age of 2; there were numerous mild attacks, usually related to respiratory infection, easily controlled, and of minimal significance during the four years previous to entry.

In November 1940, at the age of 8, he experienced the onset of watery diarrhea, with 2 to 3 bowel movements per day. There was no recognized food intolerance, and the diarrhea persisted intermittently until the patient's first admission here in 1942. In January 1941, at the age of 8½, and approximately six months after the onset of diarrhea, he had the first of a series of convulsions which led ultimately to a diagnosis of hypocalcemic tetany and to treatment with supplementary calcium and vitamin D in September 1941. He was referred to this hospital and first admitted in September 1942, at the age of 10.

Examination revealed a thin but otherwise well developed and normal boy, except for poor dentition and a Chvostek sign, both of which have persisted to the present. His clinical course and all studies since then centered around gastrointestinal-tract disturbances, parathyroid gland function and, more recently, adrenocortical function. These will be discussed individually.

Routine blood counts, serologic reactions, and chest x-rays films were repeatedly normal. Repeated determinations showed the basal metabolic rate to range from −9 to −21 per cent. The 24-hour radioactive iodine tracer uptake by the thyroid gland was 14 per cent on January 3, 1950 and 31 per cent on August 27, 1952 (normal range). Secondary sexual characteristics were normal.

Stool cultures made on his first admission revealed the persistent presence of *Salmonella panama*, which was thought probably to be responsible for the recurrent episodes of diarrhea. Attempts to eradicate this organism (which was easily grown in all stool cultures from 1942 to 1949) with sulfaguanidine, sulfadiazine, and streptomycin were completely futile. In 1949, however, following a fifteen-day course of Chloramphenicol, the *S. panama* disappeared from stool cultures and has not reappeared in 22 cultures since that time. With the clearance of *S. panama* from the stools, the patient's intermittent diarrhea almost completely subsided. Nevertheless, he continued to have occasional foul, bulky stools, as on occasions before Chloramphenicol therapy, and stool-fat studies continued to show a moderately high ratio of fat to total solid[1]. This apparent steatorrhea may well have precipitated the first manifestations of hypoparathyroidism, by its undoubted tendency to aggravate hypocalcemia. However, it cannot, of itself, explain the hypocalcemia, in view of the coexistent hyperphosphatemia (described subsequently).

[1] In 1942, total stool fat was 32.2 per cent of the solids (normal, 10–25 per cent)—24.4 per cent fatty acids and 7.8 per cent neutral fat. In 1950, total stool fat was 38.8 per cent of the solids—29.7 per cent fatty acids and 9.1 per cent neutral fat. The 1950 values were obtained with the patient receiving a diet of protein 166 Gm., fat 206 Gm. and carbohydrates 371 Gm. per twenty-four hours, and with a total stool weight of 129 Gm. per twenty-four hours.

Other studies of the gastrointestinal tract revealed: normal appearance by x-ray examination, except for slight intestinal hypomotility; normal results from a vitamin A tolerance test in 1946, and from a glucose tolerance test in 1949; normal pancreatic ferments (1946 and 1950) except for low (60 per cent of normal) amylase in 1950. *S. panama* was cultured from duodenal drainage in 1946. No crystals were seen in duodenal drainage (1948). Gastric free acid was normal in amount in 1950. A cholecystogram in 1946 showed nothing abnormal, but in 1952 it revealed many small biliary calculi (described later). A stool culture for fungi in 1952 gave positive results only for *Candida parapsilosis*.

Hypofunction of the parathyroid glands was established from the following data collected during the ten-year period, 1942–52: serum calcium concentrations[2] of 4.1–9.8 mg. per 100 ml., with a usual range of 6–8 mg.; serum inorganic phosphorus levels of 5–12 mg. per 100 ml., with a usual range of 7–9 mg.; normal renal function as demonstrated

TABLE 1. EFFECT OF PARATHYROID EXTRACT ON URINARY PHOSPHORUS EXCRETION

(Ellsworth-Howard test, Dec. 27, 1950)

Time (hrs.)	P excreted (mg. per hr.)
1	22.4
2	16.2
3	19.5
200 units of parathyroid extract intravenously	
4	49.1
5	71.4
6	38.2

by normal results from urinalyses, blood urea nitrogen determinations, intravenous pyelography, and tests of concentrating ability; and normal findings from x-ray examination of the bones (including skull[3] and long bones). The serum alkaline phosphatase level was repeatedly in the high range (5–11 Bodansky units), but might be considered normal for a growing boy (6). An Ellsworth-Howard test (1950) revealed a normal urinary phosphate response to parathyroid extract (Table 1). Sulkowitch tests for calcium in the urine repeatedly showed results of 1 plus or 2 plus through the years, despite the low serum calcium levels, demonstrating a low renal threshold for calcium. In 1950, total urine calcium was measured as 209 mg. in twenty-four hours, at a time when the serum calcium concentration was 5.9 mg. per 100 ml.

In 1949, slit-lamp examination revealed the presence of scattered small lenticular opacities, for which reason (despite no tetany since 1941) a more determined effort was made to raise the serum calcium level. A ten-day trial of AT-10 plus supplementary calcium did not cause a significant change in either the serum calcium or serum phosphorus level—a result probably related to absorption difficulties secondary to his steatorrhea.

[2] The values above 8 mg. per 100 ml. were obtained when the patient was receiving calcium and vitamin D therapy.

[3] X-ray examination of the skull shows some suggestion of calcification in the basal ganglia.

He was subsequently maintained on vitamin D_2 (Drisdol tablets) in varying doses up to 400,000 units per day, calcium gluconate in a dose of 12 Gm. daily, and aluminum hydroxide gel (Basogel), 100 to 150 cc. per day. During this regimen, serum calcium and phosphorus levels remained in the range of 7–9 mg. per 100 ml.

In the summer of 1952, because of a persistently low serum calcium level, the dosage of vitamin D_2 was gradually raised to 800,000 units per day. The patient continued his usual habit of checking urinary Sulkowitch reactions, which continued to be 1–2 plus. Approximately two weeks after institution of this high vitamin D_2 dosage, the patient began to note progressive fatigue, weakness, malaise, abdominal pain and anorexia. After about five days he was admitted to the hospital in a state of peripheral vascular collapse. Significantly, he had not noted polyuria, polydypsia, or diarrhea during the preceding days of illness, and he had vomited only once or twice.

On admission, his blood pressure was unobtainable and his heart rate was 80. His initially normal temperature rose to 101.8° F. during the first twelve hours, and his skin and mucous membranes were dry, but there were no other significant findings except a tiny area of increased pigmentation on the gums, which had never before been noted. At the time of admission, the possibility of acute vitamin D intoxication was entertained, for the initial serum calcium level was 11 mg. per 100 ml., the highest value ever seen in this patient during his ten years of observation. It seems unlikely from subsequent studies, however, that vitamin D intoxication was involved in the clinical picture.

Other admission studies revealed the following: serum sodium concentration, 110 mEq./L.; serum potassium, 6.5 mEq./L.; blood urea nitrogen, 26 mg./100 ml.; blood sugar, 74 mg./100 ml.; and hematocrit, 57 per cent. The urinary Sulkowitch reaction was 2+; and the serum amylase was normal in amount. The patient responded clinically to standard rehydration measures, but despite large amounts of intravenous sodium chloride (over 9 Gm. per day) for ten days, the serum sodium level did not rise above 125 mEq./L. On one occasion it dropped again to 115 mEq./L., with coincidental reappearance of a state of collapse and an unobtainable blood pressure. Because of this, he was started on conventional adrenal cortex replacement therapy with desoxycorticosterone acetate (DCA) and salt, during which regimen the serum sodium level rose to normal (140 mEq./L.) in four days. With this strongly suggestive evidence to support a diagnosis of adrenocortical insufficiency, the following more conclusive data were collected: 1) serum sodium concentration again fell to below 130 mEq./L. when DCA was withdrawn and supplementary salt gradually reduced to 2 Gm. per day; 2) the 24-hour urinary 17-ketosteroid excretion did not rise above 4 mg. after forty-eight hours of parenteral adrenocorticotropic hormone (ACTH).[4] (An attempt to document an eosinophil drop during these ACTH tests was unsuccessful, due to an unexplained lack of eosinophils even in the pre-ACTH blood samples); 3) a 24-hour urinary sodium determination revealed a loss of 146 mEq./L. (urinary volume 3,000 ml.) at a time when the serum sodium level was only 115 mEq./L.; and 4) renal function was normal, by the criteria previously outlined (the initial blood urea nitrogen level of 26 mg./100 ml. fell to normal within a few days after admission, and remained there).

As noted previously, the patient's early course during this 1952 admission was characterized by fever. On his second hospital day there developed an easily palpable, slightly

[4] This was carried out the first time by using 25 mg. of ACTH intravenously over a six-hour period on two successive days, and the second time by the more standardized procedure of giving 25 mg. of ACTH intramuscularly every six hours for forty-eight hours.

tender right upper quadrant mass, consistent with a distended gall bladder, and he subsequently became mildly icteric with a serum bilirubin concentration of 3.4 mg./100 ml. Blood cultures gave negative results. The fever, icterus, and abdominal mass subsided in from two to three days on terramycin therapy, but a cholecystogram subsequently revealed numerous small biliary calculi (not present in 1946), and it seemed probable that the entire eipsode of manifest adrenal insufficiency may have been precipitated by a somewhat atypical attack of acute cholecystitis.

The patient was discharged in October 1952, on a regimen of cortisone, 12.5 mg. twice daily by mouth, and enteric coated sodium chloride, 8 Gm. daily by mouth as a dietary supplement. He was readmitted six weeks later because his serum sodium level had again gradually fallen to 129 mEq./L., he had lost 4 pounds (from 114 to 110), and mild weakness had again developed. At present he is being maintained on the foregoing regimen, with the addition of DCA, 3 mg. intramuscularly twice weekly.

Finally, a word should be said about the status of his hypoparathyroidism since the onset of adrenal insufficiency. It has been a source of great interest to us, that for the first time in the ten years he was under our care, serum calcium and phosphorus levels were normal for a period of over four weeks after the adrenal insufficiency became manifest, without any supplemental dietary calcium and without any further vitamin D therapy. Recently, the former status of a serum calcium level below 8 mg./100 ml. and a serum phosphorus level above 6 mg./100 ml. has recurred, and effective control has been maintained with supplementary "Vitamin D_2 in propylene glycol" (approximately 100,000 U.S.P. vit. D units/day) and calcium lactate orally.

DISCUSSION

There are too many variables in this case to warrant drawing any exact conclusions, but it raises questions and possible interrelationships which deserve comment.

With regard to the etiology of hypoparathyroidism, it can be stated simply that there are no known causes for the cases which do not result from surgical ablation of the glands. To our knowledge, tuberculosis (which is a common cause of Addison's disease) has not been implicated in hypoparathyroidism, although it is impossible to rule it out, inasmuch as there have been no autopsies in most of the reported cases of hypoparathyroidism. There is no evidence to suggest that the patient reported here had tuberculosis at any time.

It is particularly interesting that this patient's hypoparathyroidism (as judged by serum calcium and phosphorus levels) apparently subsided for a period of over four weeks immediately following the onset of his symptoms of adrenal insufficiency. It is possible that this change in calcium and phosphorus was entirely related to the very large doses of vitamin D which he had been receiving during the previous three weeks, but there are several reports in the literature (3, 7, 8, 9) which suggest a possible calcium-lowering action of the adrenocortical steroids. The evidence for this action is still far from conclusive, and there is no adequate explanation of its mechanism, but it seems worth mentioning in view of the events of this

case. It is hoped that future studies on this patient may help to solve the problem.

This case also demonstrates the low renal threshold for calcium which Albright (6) has noted to be an occasional associated finding in idiopathic hypoparathyroidism. The nature of the renal defect is not clear; in our patient there was no other demonstrable renal abnormality.

One final point of interest is the possible interrelationship between the patient's *Salmonella panama* infection and his other disorders, particularly the steatorrhea and, more recently, the gall stones. The latter may well have been secondary to *S. panama* biliary-tract infection, in view of the earlier positive duodenal-drainage cultures.

SUMMARY

A patient with idiopathic hypoparathyroidism in whom adrenocortical insufficiency subsequently developed, is reported. Associated findings were: mild steatorrhea; a *Salmonella panama* infection of the gastrointestinal tract which responded to treatment with Chloramphenicol; biliary calculi; a low renal threshold for calcium; and an apparent subsidence of the hypoparathyroidism for a period immediately following onset of the adrenal insufficiency.

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SUMMARY REPORT OF THE 1952 WORLD HEALTH ORGANIZATION CONFERENCE ON ENDEMIC GOITER*

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A MEETING for the purpose of considering various aspects of endemic goiter was sponsored by the World Health Organization in December 1952. The study group of physicians and other interested persons[1] met at the National Institute of Medical Research, Mill Hill, London. The objectives, which were initially formulated by the chairman, Dr. F. W. Clements, were to define the magnitude of the problems from the Public Health point of view, to make a statement of what is known and what is not known of the disease and its prevention, and to suggest profitable lines of future inquiry. The details of the deliberations will be made available in a WHO bulletin. This summary report is prepared to acquaint the general reader with the findings of the study group, and particularly to point out the considerable gaps in our present knowledge.

The meeting was opened by Sir Charles Harington, who welcomed the group in behalf of the National Research Council and spoke of his long interest in problems of the thyroid gland.

PUBLIC HEALTH ASPECTS

Endemic goiter is primarily a problem of the economically underdeveloped areas of the world. Although it is one of the easiest of all diseases to prevent, it continues to be a major public health concern where ignorance, lack of interest or appreciation, or poverty has prevented the application of simple prophylactic technics. The prevalence of the disease is

Received for publication April 2, 1953.

* This paper was also published in the Chronicle of the World Health Organization, vol. 7, pp. 61–68, 1953.

[1] The members of the study group were: Dr. R. Burgess, Geneva, Switzerland; Dr. L. E. Campbell, Rome; Dr. F. W. Clements, Australia; Dr. S. Gilder, Geneva; Dr. J. C. M. Holman, London; Dr. F. C. Kelly, London; Dr. O. P. Kimball, Cleveland, Ohio; Dr. J. Matovinovic, Rebro, Yugoslavia; Prof. M. Murray, London; Dr. J. Nicod, Lausanne, Switzerland; Dr. E. E. Pochin, London; Dr. A. Querido, Leiden, Holland; Dr. V. Ramalingaswami, Coonoor, South India; Dr. H. M. Sinclair, Univ. of Oxford; Dr. R. Stacpoole, Mexico City; Mr. S. Taylor, London; Dr. B. T. Towery, Nashville, Tennessee; and the author.

rarely realized until a careful survey has been made. An entirely satisfactory goiter map of the world, therefore, cannot be drawn, but the principal zones of high endemicity are known and are illustrated in Figure 1.

It was not within the scope of the conference to discuss the problem

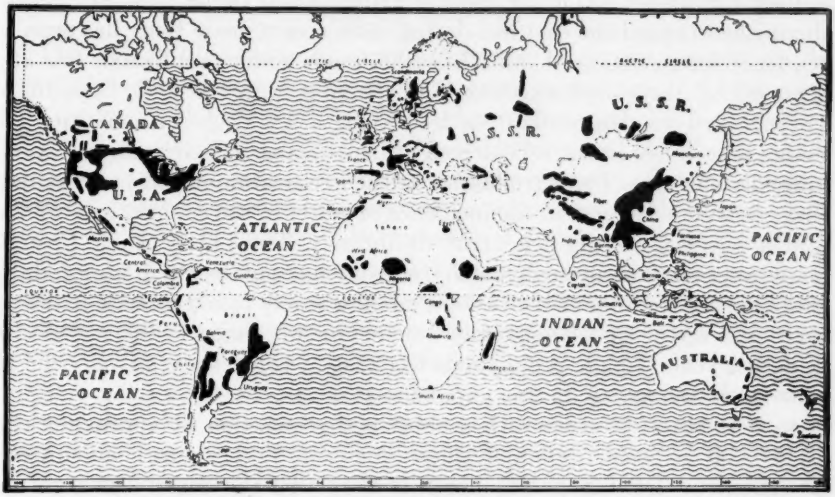

FIG. 1. The world distribution of endemic goiter
(Chilean Iodine Education Bureau, London.)
(*Bull. World Health Organization* Vol. 9, No. 2.)

in each individual endemic area, but it was possible to consider certain specific areas in detail. The situation in India was described by Dr. V. Ramalingaswami. The entire northern portion of the subcontinent is an area of intense endemicity. The belt extends across northern India into Assam. In some Himalayan villages nearly 70 per cent of the population may be affected, and even 60 per cent of infants at the breast may have definitely enlarged thyroid glands. The incidence has changed but little since the early years of this century, when Sir Robert McCarrison made his careful studies. The declining interest in the Indian endemic has been illustrated by the very few publications in the Indian medical literature which have dealt with the problem during the past few years.

Dr. O. P. Kimball sketched in the history of goiter prophylaxis in the United States from the days when he and David Marine initially began their attack on the disease in Ohio. He reported the results of many studies which showed the efficacy of iodide prophylaxis and pointed out several interesting situations in which the substitution of a highly refined salt for crude salt precipitated endemic goiter. The salutary effects of iodide pro-

phylaxis have been particularly apparent in Michigan, where compulsory iodinization of salt has been coupled with painstaking survey work.

Prof. J. L. Nicod gave an historical account of thyroid disease in Switzerland. The first goiter map of Switzerland was drawn in 1893, when the highest prevalence was in the areas of Fribourg and Berne. The prevalence diminished toward the east but during more recent years there has been a tendency for an eastward shift. In earlier years goiter prophylaxis was attempted by the simple expedients of hanging from the neck an iodine-impregnated wooden pendant, or by leaving a few drops of a solution of iodine in a saucer in the schoolroom or bedchamber. These measures were allegedly effective. The introduction of the iodinization of salt has resulted in a gratifying decrease in the incidence of goiter in the country as a whole and a decrease in the attendant disabilities. The phenomenon of jod-Basedow (iodide-induced hyperthyroidism) has not resulted from iodide prophylaxis. Nodules of the thyroid are now rarely found in male patients below the age of 35, whereas they were commonly found in this age group in earlier years. Iodide prophylaxis has been in vogue since anyone who is now below the age of 35 was an adolescent.

In 9 states of Mexico with a combined population of approximately 13,000,000 the incidence of goiter is 19 per cent. These figures, which were presented by Dr. Stacpoole, illustrate very well the control problem in Mexico. In goitrous areas cretinism is common and there is a high prevalence of deaf-mutism and mental deficiency. In the past ten years there has been a population increase of 14 per cent in nongoitrous areas, but of only 5.4 per cent in goitrous areas. All prophylactic technics which have been tried have been successful; the problem has been that of extending them to the whole population. At present there are facilities for iodinating only about 25 per cent of the salt needs of the country.

Goiter is exceedingly common in Yugoslavia, where it exists in a wide belt running diagonally along the length of the country. The disease is most prevalent in Bosnia and Slovenia. Some areas of the country are so goitrous that a residence of only six months is sufficient for the development of visible thyroidal enlargement. Prophylaxis began in 1937 and today 6 per cent of army recruits are found to be goitrous. Much of the salt is produced crudely by evaporation in pans. Iodinization of salt is now required by governmental decree.

Endemic goiter exists in several areas of New Guinea and Australia. Dr. Clements described observations on the efficiency of iodide in reducing the size of established goiter. There was a reduction of only 20 per cent in children with goiter below the age of 12, and no change at all in females between 15 and 18 years of age. The effect of iodide, therefore, is primarily preventative. Intensive surveys in Tasmania have failed to disclose dietary

differences between goitrous and nongoitrous regions. In general, routine prophylaxis with iodide has been entirely successful, but in 2 areas of Tasmania no change in goiter incidence occurred. No reason for this was apparent.

GOITER SURVEYS

The lack of uniformity in survey work has hampered progress in the prevention of endemic goiter and has retarded knowledge of the extent of the disease. Although there can be no doubt that a moderate increase of thyroid size may occur without visible enlargement of the gland, for the purposes of survey work and for assessing the intensity of an endemic the smallest goiter which should be reported as abnormal should be one which is detectable by inspection. The presence of nodules affords further evidence of the existence of endemic goiter. In conducting a survey, line drawings, diagrams, or pictures for the members of the survey teams are most helpful in gaining uniformity in classification. The single most important datum is the incidence of visible enlargement of the thyroid gland. In areas of low endemicity the most sensitive index of thyroidal enlargement is found by studying adolescent females. In a more intense endemic, adolescent males will also be involved, and when the endemic is very severe even pre-adolescent children and infants can be surveyed with profit. Unfortunately, in many surveys inadequate attention has been given to age and sex distributions. Such data are invaluable in assessing not only the extent of the endemic but the effectiveness of the prophylactic program. The study group strongly recommended that such information be included in all subsequent surveys.

SOCIAL IMPACT OF ENDEMIC GOITER

If the presence of thyroidal enlargement were the only consequence of endemic goiter the problem would resolve itself into one of esthetics. However, other defects have been attributed to the iodide-deficient state. Endemic cretinism which accompanies endemic goiter has always been a major medical and economic problem in goiter areas. The consequences of surgery may not be inconsiderable when this form of therapy has been the predominant method of dealing with the disease. There is evidence suggesting that there may be an increased prevalence of hyperthyroidism and of carcinoma of the thyroid in goitrous districts.

A much more controversial issue is whether the impact of endemic goiter can be read in terms of the prevalence of feeblemindedness, deaf-mutism, short stature, and economic unproductiveness. These defects have been duly recorded in many goiter surveys. The validity of the causal relationship is of paramount importance. The study group was unimpressed with

the reliability of much of the pertinent data. The measurement of mental competence and deaf-mutism by strangers in an undeveloped and native population may be extremely difficult. Many of the findings which have been described may be attributable to association rather than to cause and effect. The study group therefore stressed the need for further data designed to clarify the possible role of endemic goiter in depressing the level of physical and mental development of the community. Data on height, span and skeletal age, as well as mental proficiency would be highly desirable. The problems involved in obtaining such highly refined information are evident, but suitable employment of sampling technics can give data of high reliability even when small numbers of persons are tested.

IODINIZATION OF SALT

The addition of small quantities of iodide to salt is usually the most economical and effective way of supplying iodide to the people. This technic has been successfully employed in the economically better developed areas of the world where salt is distributed in a dry state and where mass methods of production have been in vogue. In other countries, programs of iodinization have met with major difficulties. For example, in India and Mexico a large proportion of the salt is produced by evaporation in pans and is distributed without further refinement. Iodinization must be performed by hand, a laborious process at best. In other areas of the world, crude salt is simply scraped from the surface of salt flats and distributed without further refinement. In still other places, climatic conditions are such that iodinated salt tends to lose much of its iodide content.

Large quantities of salt can be iodinated with extremely simple apparatus. One inexpensive device was demonstrated to the study group. It consisted of a worm conveyor which moved salt from a hopper along a trough into a receptacle. The iodide or iodate was added in the dry state to the trough by gravity from a precision feeder. One machine could supply the salt needs of several hundred thousand people.

STABILITY OF IODIDE

The stability of iodized salt depends upon the following conditions: (a) moisture and humidity, (b) light, heat and other meteorologic factors, (c) impurities in the salt, (d) the reaction of the mixture, and (e) the form in which the iodine is present. Moisture is probably the most important variable. It not only causes a loss of iodide from the salt but also a redistribution of iodide within the container. Thus if the salt is damp, higher concentrations of iodide are found in the bottom and in the top of the sack, whereas the middle zone may be relatively free of iodide. A very consider-

able collection of iodide may be found within the container and indeed, at times, almost all of the iodide can be recovered from the container. Sunlight and heat cause severe losses of iodide, and if the reaction is acid there is far more loss than if an alkaline stabilizer has been added. However, if the salt is packed dry and kept dry and if it is contained in an impervious container, its iodide content will remain stable and the distribution will remain uniform for many months. When these conditions cannot be met, it would be desirable to employ some form of iodine other than sodium or potassium iodide. Certainly the most promising substitute is iodate.

THE IODATE PROBLEM

Iodate possesses certain inherent advantages over iodide for the iodinization of salt, the principal one being stability. Under adverse conditions of moisture, heat and sunlight the iodine content of iodated salt remains relatively constant.

The pharmacology of iodate was reviewed by Dr. Margaret Murray. Earlier studies indicated that very large doses of potassium iodate are toxic to rabbits. Diffuse pigmentary changes which were reminiscent of retinitis pigmentosa were seen in the ocular fundus. The quantities necessary to produce these changes were far in excess of those which would conceivably be used in the iodinization of salt. Dr. Murray also reported briefly on certain studies carried out by Dr. Perry of the National Institute of Medical Research. He found that sodium iodate has a very low order of toxicity for mice and rabbits. No pathologic changes were detected in the retina or kidneys of these animals. These studies suggest that salt can be iodinated with iodate with perfect safety at a level of 1 part in 1,000, which is a concentration far higher than would be necessary for the prevention of endemic goiter.

Certain physiologic studies with iodate were reported by Dr. E. E. Pochin. Employing I^{131}-labeled iodate, Dr. Pochin was able to show that almost all of the iodine of iodate is available for utilization by the thyroid gland. Although his results did not prove that the iodine which is derived from the ingested iodate is actually formed into hormone by the thyroid gland, the presumption would be that this iodine is actually available for hormone synthesis.

The study group concluded that it is safe to use iodate interchangeably with iodide for the iodinization of salt, that it is effective, and that it has certain advantages of stability over iodide. Insufficient studies have been made at the field level for a definite conclusion regarding the effectiveness of iodate in the prevention of endemic goiter. Preliminary results suggest that it is equally effective.

LEVEL OF IODINIZATION OF SALT

The desirable concentration of iodine in salt depends upon the daily iodine requirement and the daily salt intake. The latter varies widely. For example, children may take no supplementary salt except that which may be used in cooking. On the other hand, the average daily consumption for hot and humid countries such as southern India, may approach 30 grams.

The recommended iodide content of salt has varied widely. In the United States it is 1 part in 10,000, whereas in Switzerland it has been 1 part in 200,000. Fortunately, the thyroid gland is capable of adjusting to wide variations of iodine supply.

The study group recommended that salt be iodinated to a level sufficient to supply 100 micrograms of iodide daily, or its equivalent quantity of iodate. The concentration of iodine in the salt must, therefore, depend upon the salt habit of the people of the community. A considerable excess of iodide is tolerated.

IODIDE REQUIREMENTS AND THE PHYSIOLOGY OF THE IODIDE-DEFICIENT THYROID GLAND

Only meager information is available regarding the metabolism of iodide by goitrous patients in an endemic area. Data were presented from observations in western Argentina. Many of the subjects of this study were found to have thyroid glands which had a very high avidity for, and a very rapid turnover rate of iodide. There appeared to be an inverse correlation between the quantity of iodide in the diet and the I^{131} uptake by the thyroid gland. Those patients who had adequate dietary iodide had low uptakes of I^{131}, whereas those with inadequate dietary iodide had high uptakes of I^{131}. Most of the patients who were excreting less than 40 micrograms of iodide daily had I^{131} uptakes of greater than 60 per cent.

When daily supplements of iodide were given to some of these patients there was a slow fall in the avidity of the gland for iodide, and there was an accompanying positive iodide balance. In 1 patient, who was given 1,500 micrograms of iodide daily for six weeks, thyrotoxicosis developed. Studies of the effects of varying quantities of added stable iodide on the uptake of radioactive iodide showed that the uptake is little influenced by single doses of iodide below 2 milligrams, but when a single dose of more than 5 milligrams is given, most of it is wasted through the kidney.

These studies, together with others, suggest that the daily intake of iodide which is adequate for the prevention of endemic goiter is 100 micrograms. This allows for a certain margin of safety and is far below the level which has given any toxic effects. These data also suggest that in prophylactic programs in which iodide is given intermittently in large doses, as

in iodate candy, it is uneconomical to give more than 5 milligrams of iodide in any single dose.

NEED FOR FURTHER INVESTIGATIONS

Throughout the deliberations of the study group it was repeatedly evident that there is need for further information on many aspects of endemic goiter. Some of these deficiencies have already been implied. Among the others are the following:

1. The world distribution of endemic goiter needs to be more carefully mapped.

2. The relationship of endemic goiter to iodide deficiency needs to be more clearly defined. This can be accomplished when appropriate facilities are available for measuring the urinary excretion of stable iodine, which is a helpful measure of iodide intake.

3. Although iodide deficiency is certainly the cause of most endemic goiter, valuable information may derive from dietary and nutritional surveys in relationship to endemic goiter.

4. There is need for more careful study of the relationships of endemic goiter to feeblemindedness, deaf-mutism, and retardation of growth.

5. Valuable information may result from further studies of the metabolism of iodide by the iodide-deficient patient. These studies should include, when possible, the use of radioactive iodide and of chemical iodide assay. It would be particularly valuable to observe with these technics the long-term effects of supplemental dietary iodide.

6. Development of simplified methods for the determination of iodine in biologic fluids would be invaluable in field studies of endemic goiter.

7. There is sufficient evidence from the older literature to warrant reinvestigation of the relations between hardness of water, calcium intake, and endemic goiter. These studies could be relatively simple in a laboratory equipped for the determinations of I^{127}, I^{131}, and calcium.

8. There is confusion regarding the relationship of endemic goiter to carcinoma of the thyroid. Properly performed studies made with reference to histologic types of thyroid tumors should provide valuable information concerning the relationship of tissue hyperplasia to neoplasia.

9. The pathogenesis of thyroid nodules could be studied to advantage by the technics of radioautography in an area of endemic goiter.

10. Further information is needed regarding the effects of storage, temperature changes, moisture, and handling on the stability of salt iodinated with iodide and with iodate.

11. There exists almost no information regarding the stability of iodide and iodate in the processes of cooking.

CONCLUSIONS

1. Whenever possible, salt should be iodinated at a level which will provide approximately 100 micrograms of iodide daily to the consumer.

2. When it is economically impractical to supply the entire population with iodinated salt, careful surveys should delineate the areas where endemic goiter exists. Iodinization should be legally mandatory.

3. Iodate can be used interchangeably with iodide and has certain advantages in stability under conditions of heat and moisture.

4. There exists a need for further investigation of the nature and impact of endemic goiter, of the physiology of the iodide-deficient gland, and of the technics for production and distribution of iodinated salt.

Letters to the Editor

ESTRADIOL CYCLOPENTYLPROPIONATE: A NEW, LONG-ACTING, INJECTABLE ESTROGEN

To the Editor:

Recently a new, long-acting, injectable estrogen, estradiol cyclopentylpropionate,[1] has become available for evaluation. Studies in spayed rats (1) have been made, comparing the duration of action of estradiol cyclopentylpropionate and estradiol dipropionate. Following a single subcutaneous injection of 10 mg. of the estrogen in cottonseed oil, it was found that on the fiftieth day after the injection only 10 per cent of the animals receiving the dipropionate were in estrus, whereas 70 per cent of the animals given the cyclopentylpropionate were in estrus. In view of this prolonged activity of estradiol cyclopentylpropionate in rats, it appeared desirable to study the preparation in the menopausal female.

Results. In this study, 253 menopausal patients have been followed for more than one year under estradiol cyclopentylpropionate therapy[2] combined with varying dosages of testosterone cyclopentylpropionate. In general, it was found that a single injection of from 1 to 3 mg. caused relief of menopausal symptoms for periods of three to six weeks. No untoward side effects of either a local or systemic nature were observed, when given with testosterone cyclopentylpropionate. The drug was well tolerated locally and did not cause any irritation at the injection site in either concentration used (1 mg. or 5 mg. per cc.). Vaginal bleeding (withdrawal or breakthrough) did not occur in a single instance in the series of 253 cases when conbined estrogen-androgen therapy was used.

Discussion. The chief advantage of a long-acting, injectable estrogen or androgen is the excellent control which it gives the physician over the condition of the patient. There is a marked variation in females as to their tolerance of testosterone compounds.[3] In the series observed, most pre-

[1] Depo-Estradiol, Upjohn.

[2] The drug was supplied in two concentrations, 1 mg. per cc. and 5 mg. per cc., in cottonseed oil, by the Upjohn Company, Kalamazoo, Michigan.

[3] Many of these females tolerated 100 mg. of testosterone cyclopentylpropionate monthly along with from 2.5 to 5 mg. of estradiol cyclopentylpropionate without any manifestations of virilization. However, some females show noticeable hirsutism on dosages as small as 25 mg. monthly. From my observations of the combined use of estrogen-androgen mixtures in the treatment of the menopause I am satisfied that no manufacturer will be able to produce a fixed dosage of combination estrogen and androgen that will be satisfactory for general use, because of the extreme variance of sensitivity to testosterone or estrogen.

viously untreated women with marked menopausal symptoms were started on a regimen of 2 mg. of estradiol cyclopentylpropionate and 100 mg. of testosterone cyclopentylpropionate given in one injection every four weeks. If there was any manifestation of beginning virilization, the dosage of androgen was decreased and the estrogen increased. Any patient receiving potent endocrine therapy should be seen by the physician about once a month. Since an injection of estradiol cyclopentylpropionate lasts about four weeks, the treatment schedule coincides conveniently with the follow-up schedule.

Although oral drug therapy has certain advantages, it also has some disadvantages. Many women, even if apparently of the intelligent type, feel if one pill a day makes them feel better, two a day would make them feel twice as well. It is not uncommon for a pharmacist to continue refilling potent oral estrogen prescriptions without authorization or notification to the prescribing physician. Withdrawal or break-through vaginal bleeding is a common complication of unsupervised oral estrogenic therapy.

Summary. Estradiol cyclopentylpropionate is an exceedingly potent, long-acting, injectable estrogen. In a dose of from 1 to 3 mg. it gives symptomatic relief of the menopausal syndrome for a period of from three to six weeks.

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DETERMINATION OF 17,21-HYDROXYCORTICOSTEROIDS IN PLASMA*

TO THE EDITOR:

When Nelson and Samuels (1) published the method for the determination of 17,21-hydroxycorticosteroids in blood plasma, a number of important details were not given. Some were omitted in order to save space; the importance of others was not recognized at the time. Since many workers who are trying to use the method are encountering difficulty because of neglect of these details, it seems important to publish them so that other workers may avoid such pitfalls.

* This investigation was supported in part by research grants A-29 from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service, in part by the Medical Research and Development Board, Office of the Surgeon-General, Department of the Army, under contract No. DA-49-007-MD-36, and in part under a research grant from the American Cancer Society.

A. *Preparation of blood plasma:* Heparin is preferable as an anticoagulant, since a hazy solution is occasionally obtained in the final reaction when citrate is used. This is probably because traces of the calcium salt are carried through the solvent systems and precipitate as calcium sulfate in the strong sulfuric acid.

The blood should be centrifuged immediately after drawing the sample, in order to separate cells and plasma. Normally, the peripheral plasma contains at least 90 per cent of the compounds being measured, but if blood is allowed to stand the cells gradually lose their differential permeability and the steroid begins to penetrate. If extraction of the plasma cannot be performed immediately, it is advisable to store the sample in a deep-freeze.

B. *Chloroform extraction:* To prevent destruction of the steroids by traces of phosgene, the chloroform for extraction should be used within four hours after distillation. Since light of shorter wavelengths in the visible spectrum can readily promote formation of small amounts of phosgene in chloroform, which will destroy significant proportions of the fraction of a microgram of steroid ordinarily being worked with, the distillation of chloroform should not be carried out in direct sunlight or under illumination with fluorescent light. The chloroform should be stored in brown bottles and after the extraction has been made as previously described, the chloroform solution should not be exposed to sunlight or direct fluorescent light during the subsequent evaporation. The temperature of the bath in which the evaporation is carried out should not be above 45° C.

C. Preparation of Florisil:

Soak the dry Florisil (obtained from the Floridin Company, Warren, Pennsylvania) in 95 per cent ethanol for from twelve to fifteen hours, being sure the Florisil surface is completely covered. Stir once every hour for the first six to eight hours. Filter the ethanol from the Florisil and rinse the latter with U.S.P. absolute ethanol (2,000 ml. to 500 Gm.). Filter off the ethanol as completely as possible. Transfer the Florisil to a porcelain dish and dry it at 120° C. for twenty-four hours; then activate at 600° for four hours. Allow the Florisil to cool down to room temperature in the muffle-furnace and immediately transfer it to glass-stoppered flasks which are kept in a desiccator over silica-gel.

The Florisil so prepared and stored can be used over a four-day period. If kept longer, it is advisable to repeat the dehydration of the Florisil by heating at 120° C. overnight. Apparently, anything more than a trace of moisture adsorbed on the column causes significant loss of steroid during the chromatography. Originally this was not appreciated in our laboratory because of the low humidity of the region. The protection of the Florisil from adsorption of moisture is very important in regions of relatively high humidity.

D. *Preparation of glass wool:* The glass wool used for supporting the Florisil column can be an important source of contamination. Pyrex-glass wool has been found to be the best, but it needs thorough cleaning. The glass wool should be soaked in redistilled chloroform for one hour with occasional stirring. This chloroform should then be poured off and the procedure repeated. After the second washing the glass wool is allowed to dry at room temperature and is then ready for use. Obviously, it is important in handling the wool and all other reagents and apparatus that oil from the skin should not be introduced into the system; otherwise, it will give a brown color with the sulfuric acid reagent and add to the blank.

E. *Washing the reaction flask:* When one is extracting such minute amounts of material with relatively large volumes of solvent it is important to wash very carefully the sides of all flasks in which solvent is evaporated. This is particularly true in washing down the sides of the reaction vessel after the 25 per cent methanol-chloroform fraction collected from the chromatographic column has been evaporated to dryness. Since the volume of washing fluid should be less than the capacity of the well in the bottom of the flask, it is necessary to use a very fine jet forcibly sprayed over the entire surface. For this purpose we have found it convenient to use a 24-gauge needle bent at an obtuse angle, attached to a Luer-Lok syringe filled with washing solvent. This is rotated in the neck of the flask so that the entire surface of the bulb is washed with a small volume of solvent. The washing must be repeated at least three times, the solvent being evaporated to dryness after each washing.

If the precautions set forth here are heeded, in addition to the details of procedure given in the original report, analytical results as good or better than those set forth in the original publication can be expected. Like all micro procedures, however, good results cannot be expected in the hands of inexperienced and clumsy technicians.

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Teaching Clinic

ANTERIOR PITUITARY INSUFFICIENCY FOLLOWING SKULL FRACTURE

REPORT OF A CASE

The occurrence of Simmonds' disease following trauma is a rare clinical condition. Escamilla and Lisser (1) who, in 1942, analyzed 595 cases, 101 of which were proved pathologically, quote only 4 cases in which the condition was due to trauma. Three of these had been previously reported in the medical literature (2–4) and there was a fourth which was the subject of a personal communication to the authors. Other patients with Simmonds' disease following trauma include: one reported by Schereschewsky (5), one by Berglinger (6), two by Lerman and Means (7), one by Lisser and Curtis (8) and one by Robertson and Kirkpatrick (9), bringing the total to 10 cases reported to date, to our knowledge.

Because of the rarity of the condition we wish to add another case recently observed by us.

CASE REPORT

A 25-year-old man was admitted to the Clinic on October 16, 1951. In January 1948 he had been caught behind a truck which backed into him, compressing his head against a brick wall. He suffered a skull fracture, bled from ears, nose and mouth and was semiconscious for two weeks. The right side of the face was paralyzed and he could not open his right eye, which remained shut for about a year and then gradually opened.

Two months after the accident he noticed a gradual loss of axillary and pubic hair and a decrease in the size of his testes; there was also impotence and loss of libido. He needed to shave only every three months, whereas before the accident he shaved three times a week. At this time he was informed by his doctor that he had low blood pressure and was anemic. He tired easily, which prevented him from working for two and a half years, and also suffered from dizziness, nausea and vomiting on exertion.

At the time he was seen in the Clinic the impotence, decreased libido and easy fatigability persisted, and he still felt dizzy and nauseated after exertion, though these symptoms had improved somewhat. He could not move his right eye and suffered from diplopia.

Family and personal histories were noncontributory.

Physical examination revealed a man 5 feet 5 inches tall, weighing 155 pounds, with a pulse of 80 per minute and a blood pressure of 80/60. There was no pubic or axillary hair and almost no beard (Fig. 1). There was no abnormal pigmentation. The testes and prostate were small and soft and the penis of normal size, though the patient thought that it was smaller than before the accident. There was an appearance of enophthalmos on the right with internal strabismus O.D. and anisocoria, and neurologic examination showed paralysis of the right fifth, sixth and seventh nerves.

Laboratory examinations revealed the following:

X-ray examination of the skull showed a radiolucent line on the left front temporal region which, when compared with an older x-ray picture taken elsewhere, proved to be the residue of an old fracture (Fig. 2); the sella turcica measured 18 mm. in its sagittal diameter with no demonstrable pathologic changes. Funduscopic examination showed a slightly contracted form field, O.D. and early optic atrophy, O.S., with no evidence of chiasmal involvement.

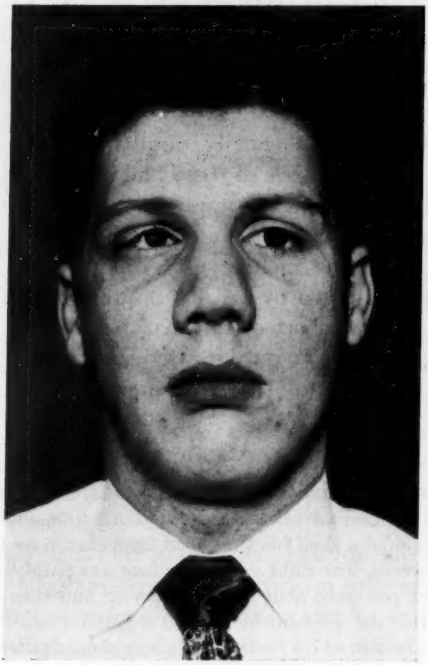

FIG. 1. Typical facies with relative lack of beard development.

Urinalysis gave normal findings. The red blood count was 4,120,000 per cc., white blood count 7,350 per cc., hemoglobin 11.0 Gm. and hematocrit 34; differential—52 per cent neutrophils, 41 per cent lymphocytes and 7 per cent monocytes. Wassermann and Kahn tests gave negative results. The fasting blood sugar level was 75 mg. per 100 ml. and a glucose tolerance test showed the following: fasting 80 mg., ½ hour 142 mg., 1 hour 157 mg., 2 hours 58 mg., 3 hours 52 mg., and 4 hours 41 mg. per 100 ml. The basal metabolic rate was −28 per cent and −27 per cent, respectively, on two occasions. The blood cholesterol level was 238 mg. per 100 ml. Radioactive iodine uptake was 23 per cent at the end of twenty-four hours. An ACTH test showed a 15.5 per cent drop in blood eosinophils after an initial count of 275. The Robinson-Power-Kepler water excretion test revealed the largest day hourly urine volume to be 44 ml. and the overnight volume, 240 ml., with an index of 10.3 (normal above 25). On three determinations the 17-ketosteroids were 0.8, 1.0 and 1.2 mg. per twenty-four hours, respectively. These measurements were made on a relatively purified neutral fraction. (Normal values in

our laboratory are from 7 to 14 mg. per twenty-four hours.) Testicular biopsy showed hypoplasia and anakmesis of the tubular epithelium, sclerosis of the basement membrane, and sclerosis of the interstitial tissue with hypoplasia of the Leydig cells (Fig. 3).

Therapy was instituted with testosterone cyclopentylpropionate (200 mg. intramuscularly per week), oral cortisone (12.5 mg. twice a day), desiccated thyroid (1 gr. daily) and iron gluconate (30 gr. daily). After nearly two months of this therapy, hormonal treatment was interrupted for a month. At the end of this period the urinary gonadotropins were less than 6 m.u. per twenty-four hours.

FIG. 2. Roentgenogram of the skull, taken a few days after the accident. Note fracture line and normal sella turcica.

Therapy was re-instituted as before, and at the end of six months the patient experienced considerable clinical improvement with a gain in weight of 15 pounds. Blood pressure varied between 100/72 and 110/75. Blood cholesterol concentration on two determinations was 289 and 291 mg./100 ml., respectively, and the basal metabolic rate was −18 per cent. The Robinson-Power-Kepler index had risen to 33; however, during the first part of the test the result was still positive, with a maximum day hourly urine volume of 55 ml. and an overnight volume of 250 ml. Serum sodium concentration was 131 mEq./L. and potassium, 5.2 mEq./L. There was no radioactive iodine uptake at the end of twenty-four hours. The patient stated that he shaved more often and there was a generalized increase in body hair. Libido and potentia were markedly increased and improvement in his energy and endurance permitted him to take a job as a tool maker.

Therapy with cortisone was discontinued and testosterone, thyroid, and iron gluconate were continued as before. Seven months after this the Robinson-Power-Kepler index was 23.0, with a maximum day hourly urine volume of 45 ml. and overnight urine

volume of 240 ml. The level of fasting blood sugar was 71 mg./100 ml.; of serum sodium, 135.5 mEq./L.; and of potassium, 3.8 mEq./L.

There has been no improvement in eye symptoms and extra-ocular muscle surgery is being contemplated.

DISCUSSION

There are several important points in the history of this patient's condition which clearly indicate pituitary damage of a severe degree. For many months after recovery from his injury, energy had remained at a very low level. The degree of fatigue which he experienced after mild exercise was

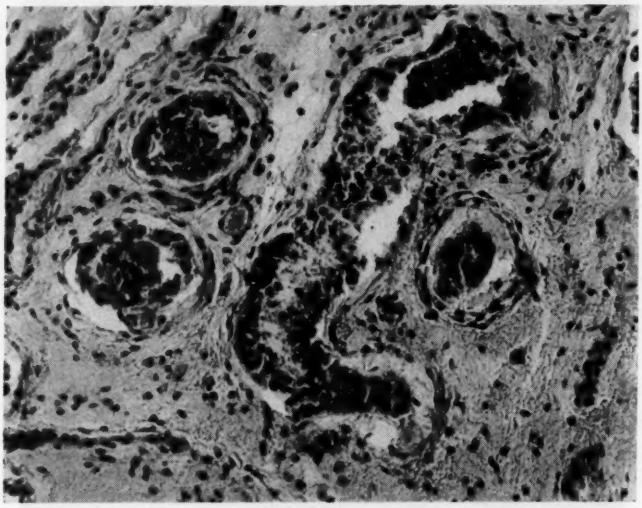

FIG. 3. Microscopic section from testis biopsy specimen, ×200. (For explanation, see text.)

comparable to that seen in Addison's disease. He experienced loss of libido, and complete impotence. These symptoms are almost always present under such circumstances. The nausea, vomiting and dizziness on mild exertion were thought to be due to the extremely low blood pressure with superimposed postural hypotension. It is not unlikely that adrenal deficiency was playing a part. Symptoms suggestive of hypoglycemia were not present; in our experience, pituitary deficiency of severe degree is not usually associated with acute symptoms of hypoglycemia as frequently as is Addison's disease. Loss of body hair was a striking physical sign which may be considered good evidence of adrenal as well as of testicular deficiency although loss of body hair does not occur to a degree which is important as a diagnostic sign of Addison's disease or of severe testicular failure alone. Growth

of scalp hair is not affected so strikingly in severe pituitary failure; but in some instances in which pituitary deficiency of a marked degree occurs following removal of a pituitary tumor, the hair may grow less than half an inch in six months after the head has been shaved in preparation for surgery.

The patient had noted decreased size of the testes. Under the circumstances this, too, is strongly suggestive of pituitary failure due to lack of gonadotropins, particularly follicle-stimulating hormone (FSH), since the testicular size is chiefly dependent on the mass of the tubules. Softness of the testes and diminution in their size is a common accompaniment of milder grades of pituitary failure, as seen in cases of pituitary tumor. Anorexia nervosa seldom occurs in men, but when it does, it represents the physiologic counterpart of pituitary failure of organic origin. Under such circumstances libido and potency are low, but in our experience there has never been a striking loss of axillary, pubic and body hair. With regard to libido it is also interesting that retention of this function may be a clue to a proper diagnosis, when pituitary failure may be suspected. For example, in women who have postpartum amenorrhea due to pituitary failure, libido diminishes and there is evidence of ovarian and adrenal deficiency; whereas libido remains normal in patients whose amenorrhea is due to hyperinvolution of the uterus.

It is interesting that there was no fasting hypoglycemia in our patient. Results of the glucose tolerance test were not particularly unusual, except that the blood sugar concentration fell to levels lower than ordinarily seen at the end of the test. This rather striking hypoglycemia is interesting and strongly suggests a hypersensitivity to insulin. A flat glucose tolerance curve is often claimed to be of diagnostic value in patients with pituitary deficiency. In our experience, this test is of relatively little value for this purpose, because flat glucose tolerance curves are seen frequently in conditions in which no pituitary deficiency exists. Insulin tolerance tests were not carried out in this patient, but it is likely that if they had been performed they would have shown a hypersensitivity to insulin. In practice we are usually disinclined to use tests which may be disturbing and in some instances dangerous to the patient, especially when the diagnosis is reasonably clear on the basis of other tests.

Hypometabolism of severe degree is a constant accompaniment of severe anterior pituitary failure; levels as low as −40 per cent are commonly seen. We have not seen a very low basal metabolic rate and clinical myxedema in a patient with severe pituitary failure, even though the radioactive iodine uptake in the thyroid may be low and the blood cholesterol level may be as high as 400 mg. per 100 ml. The fact that the thyroidal

radioactive iodine uptake in this man was 23 per cent seemed to indicate that most of the depression of his basal metabolic rate was through a mechanism which was extrathyroidal. It is also worthy of note that, after treatment, when the basal metabolic rate had risen from −28 per cent to −18 per cent, presumably largely through the use of thyroid, there was no uptake of radioactive iodine. This may indicate that prior to treatment thyroid function was being maintained to some degree by the remaining pituitary activity, and that the fall of radioactive iodine uptake was due to the suppression of thyrotropin production by the administered thyroid. Measurement of the urinary gonadotropins in this case showed low values, as would be expected. In instances in which pituitary failure is suspected, but doubt exists, this assay may be extremely useful. We depend upon it a great deal for differential diagnosis. A low level of 17-ketosteroids in the urine is also a dependable aid to diagnosis, but it is not as specific an indicator of the degree of pituitary failure as is gonadotropin excretion. An extremely low level of 17-ketosteroids is also seen in myxedema, in Addison's disease in women, in some cases of anorexia nervosa and in severe malnutrition and serious chronic illness. Some normal women excrete exceedingly small amounts of these materials.

Testicular biopsy is of interest here, because it provides an opportunity to observe changes in the testes which are almost certainly of pituitary origin. The biopsy revealed shrinking of the tubules, pronounced hypoplasia of the gametogenic elements and arrest of spermatogenesis. Testicular changes in men with pituitary failure associated with pituitary tumor have shown that the outstanding findings were diminution of testicular mass due to shrinkage of the tubules, and arrest of maturation of spermatocytes with a tendency to reversion of the gametogenic cell to forms similar to those seen during prepuberal years (10). The Leydig cells tend to diminish in number and size. The basement membrane becomes thickened. The Sertoli cells show much less effect than is seen in other cellular elements in the testes.

With regard to the tests for adrenal function, it is interesting that the first ACTH test performed in such a patient is likely to show little or no response—as was the case in the patient reported here. Repeated injections of ACTH will revive the activity of the adrenals, as measured by the eosinophil response to this hormone (11).

The Robinson-Power-Kepler water test is useful under such circumstances, and it commonly shows a highly positive result, similar to that seen in Addison's disease. In pituitary failure the test frequently gives a normal index, but in the first part of the procedure the findings are strongly positive. This was true in our patient, following treatment; the index rose from

an original level of 10 to a level of 23, but the urine volumes remained decidedly abnormal, the overnight specimen measuring 250 ml. and the highest day hourly volume being only 45 ml.

There is a significant degree of adrenal failure in these patients and it is important to take this into consideration in the event of surgery, acute illness, or some unusual stress. Under these circumstances, adrenal failure which may be latent and producing few symptoms sometimes becomes severe enough to be dangerous to the patient, unless the condition is recognized and treated with cortisone, adrenocorticotropin (ACTH), or both. When adrenal failure is of pituitary origin it is interesting that crises such as are so commonly seen in Addison's disease seldom occur, although they are not unknown (12). When such a crisis arises it may be accompanied by stupor, fever without leukocytosis, hypoglycemia, and arterial hypotension; there is a dramatic response to intravenous glucose-salt and adrenal hormone therapy. Our experience with the treatment of Simmonds' disease in men coincides with that of others (8, 13). We have found that the response to testosterone therapy alone may be so good, and the patient may be made so nearly symptom-free by its use, that little symptomatic improvement can be produced by the subsequent addition of thyroid or cortisone.

In women with this condition more dependence must be placed upon thyroid and cortisone, since the amount of testosterone that can be given without masculinization may not be sufficient to overcome the symptoms completely.

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Editorial

PSYCHOLOGIC CHANGES IN ADDISON'S DISEASE

PSYCHOLOGIC disturbances may be of etiologic significance in certain endocrine disorders as Hoskins (1) has recently emphasized in discussing thyrotoxicosis. This is a paradigm of psychosomatic disease. The psychosomatic approach must, however, include conditions which are better described as somatopsychic, in that the physical disturbance is definitely primary. Under such a heading fall the endocrine deficiency states such as myxedema, with its classic picture of retarded mental processes and the less frequently occurring but none the less definite psychosis. Close inspection reveals that the psychologic picture in myxedema is not so classically clear as to be pathognomonic and that very little systematic psychiatric assessment has been recorded. Only recently have intellectual functions in myxedema been investigted by quantitative, projective tests (2). Addison's disease belongs to the same category as myxedema, from both the endocrinologic and psychologic point of view, but does not always exhibit as striking a set of mental changes. Interest in the mental state of patients with adrenal insufficiency has grown as the result of two developments in the past decade. These are, 1) the emphasis placed by the psychosomatic approach on the importance of psychologic aberrations in a variety of diseases and 2) the recent studies indicating that there is hypofunction of the adrenal in schizophrenia. Thus, observations concerning this gland are both critical and topical.

The common mental symptoms encountered in Addison's disease have often been briefly described but have not attracted much attention from psychiatrists. They have usually been ascribed to exhaustion, anemia, general debility or some such catch-all concept. Psychoses associated with Addison's disease have been reported occasionally during the last fifty years, but have been regarded as coincidental or due to a disturbance in salt or water balance. The frequency of abnormal mental states in Addison's disease was first clearly described by Engel and Margolin (3) in 1942. They found various neuropsychiatric disorders in 16 of 25 cases seen over a ten-year period. The writer's observations are strikingly similar (4). In a series of 25 cases, apathy and negativism were encountered in four fifths, and seclusiveness, depression and irritability in about half. Various authors have described an association between the biochemical and mental devia-

tions, mental deterioration occurring in conjunction with electrolytic changes and hypoglycemia. This covariation is not invariable, however, for exceptions are met in which disturbed mental status is seen despite a normal chemical blood picture (4, 5, 6).

The first definitive comparative psychiatric appraisal of the mental status in Addison's disease was published recently by Stoll (6), who reported that the incidence of psychopathic disturbances was 35 per cent in the preaddisonian state but rose to 66 per cent after the disease developed. In a study of 29 chronic cases he described 3 categories of psychic anomalies. The first, found in acute or terminal adrenal insufficiency, was characterized by a nonspecific delirium (acute organic reaction). The second and third occurred in chronic insufficiency. The second type of reaction consisted of chronic mood anomalies, and periodic disturbances in temper, activity and general drive (endocrine or localized brain psycho-syndrome of M. Bleuler.) In fact the symptoms were neither homogeneous in kind, direction or intensity, nor did they parallel the physical findings. The situation was the opposite in the third type, which included the rare psychoses (organic diffuse brain psycho-syndrome of E. Bleuler). These three basic types were often found combined. They could be distinguished from schizophrenia, though there were certain resemblances.

The basis for the psychologic symptoms is not understood. There is no decrease in cerebral oxygen consumption (7), as there is in myxedema (8), but clinically the change in general drive may be an almost suicidal apathy or a non-achieving, aimless querulousness. Brain metabolism is altered, however, as shown by the frequent occurrence of electroencephalographic abnormalities (9) and a tendency toward the development of hypoglycemic symptoms despite a blood sugar level which is relatively high.

The introduction of cortisone has provided a means of treatment more adequate than any heretofore. Besides enabling patients to withstand stress, to undertake a normal load of work and to experience a return of vigor far beyond that achieved by desoxycorticosterone therapy, it leads to such an improvement in the psychologic status that the abnormality of thinking and feeling prior to such therapy is heavily underscored. Mental excitement often occurs early in the period of treatment with relatively small doses of the drug and transient psychoses may be precipitated (9, 10) but both these manifestations subside within a few days or weeks, to leave the patient in a much more balanced frame of mind.

Individuals with adrenal insufficiency secondary to hypopituitarism also show psychologic disturbances and these may be even more severe than those usually seen in Addison's disease, due to the associated hypothyroidism or other hormonal deficits, and occasionally to brain damage.

The severity of the psychologic complications renders the secondary type of adrenal insufficiency less suitable for the study of the influence of hormones on behavior.

Although Addison's disease is relatively rare and new cases uncommon, there are opportunities for making systematic investigations correlating biochemical and psychologic changes with therapy. Few workers have had much chance to observe the effect of cortisone in this condition. Rewarding results might reasonably be expected if clinical investigations are carefully carried out by endocrinologic and psychiatric technics prior to, and following treatment with cortisone or allied compounds.

ROBERT A. CLEGHORN, M.D.

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Questions and Answers

(Questions submitted at the 1952 Postgraduate Assembly of the Endocrine Society)

Answer by John Eager Howard, Baltimore, Md.

QUESTION: Have you tried cortisone in constitutional hypertrichoses?

ANSWER: By constitutional hypertrichosis it is assumed that the questioner means excess of body and facial hair in women with no other stigmata of masculinization—the type of thing that often runs in families, usually becomes evident at or soon after the menarche, and is more prevalent in dark skinned races. So far as we are aware, persons with these criteria have always had normal 17-ketosteroid excretion. In this group, cortisone has proven of no benefit so far as we are aware. However, in persons with hirsutism, menstrual irregularity and/or sterility, sometimes with small uteri and cystic ovaries, whose excretion of 17-ketosteroids may be slightly above the normal (say 15–20 mg. per day), there has been reported from the use of cortisone, improvement in menstrual regularity, ovulation as judged by the temperature chart, and even pregnancy (1). These people are visualized as representing minimal degrees of a functional adrenal abnormality (of which the maximal degree is female pseudohermaphrodism, which responds so well to cortisone (2)).

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Answers by Howard Root, Boston, Mass.

QUESTION: Is constitutional thinness related to insulin?

ANSWER: Constitutional thinness is not related to insulin so far as is known. Studies now
n progress suggest that diabetic patients have a constitutional stockiness, rather than
hinness.
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QUESTION: One internist felt that a patient whose urine showed 1+ sugar in only one 2-hour specimen was a prediabetic or early diabetic. Please discuss the significance of this type of blood sugar curve.

ANSWER: One plus sugar in a 2-hour specimen of urine might be considered diagnostic of diabetes, provided the blood sugar level at the same time was definitely above normal. In a diabetic a glucose tolerance test carried out with 100 grams of glucose given by mouth ordinarily would produce an abnormally high elevation of the blood sugar level at thirty minutes to sixty minutes after taking the glucose. One would expect sugar in

the urine at that time, even if the urine was sugar-free at the end of two hours. However, in a good many patients the blood sugar value may be high at the end of sixty minutes with little or no sugar in the urine, and yet at the end of two hours the urine may show a good deal of sugar when the concentration of blood sugar has already begun to fall. The diagnosis of diabetes is made by some students when the peak of the blood sugar curve exceeds 170 mg. (Folin-Wu, venous blood); and by some students, if the blood sugar has not returned to the original fasting level at the end of two hours.

QUESTION: Would you comment on the use of oral cortisone in small amounts for the prevention of insulin reactions in the severe "fragile" diabetic?

ANSWER: Oral cortisone in small amounts might theoretically be of value in preventing insulin shock in the "fragile" diabetic. However, no extensive experience is available with such cases. Having in mind the known deleterious effects of cortisone upon diabetes, it hardly seems justifiable to use cortisone in this way in such patients.

QUESTION: Is there any relationship between the cerebral pathology of insulin shock and that of epilepsy?

ANSWER: In certain patients with diabetes the development of a low blood sugar level due to insulin action is accompanied by typical epileptic seizures. This is by no means true of all patients who get insulin hypoglycemia. In certain patients with diabetes of long standing, frequent insulin reactions seem to be followed within a few years by the development of an epileptic state in which seizures may occur without hypoglycemia. In certain diabetic patients in whom insulin hypoglycemia occurs rather easily and rather rapidly, the encephalogram shows some changes in the tracing; these changes are not typical of epilepsy but, nevertheless, suggest some cortical abnormality. The relationship therefore, between the pathologic mechanism of insulin shock and that of true epilepsy seems difficult to define. It is commonly thought that repeated insulin shock may bring to light latent epilepsy, rather than that insulin shock itself will induce epilepsy anew.

QUESTION: Is diabetes a contraindication to the use of insulin shock therapy for psychosis? Is this procedure likely to produce insulin resistance that will complicate the management of the diabetes?

ANSWER: Diabetes is not necessarily a contraindication for the use of insulin shock therapy for psychosis in selected patients. However, it is true that diabetic patients who are sensitive to insulin are also vulnerable to severe hypoglycemia, so that the risk of a fatal hypoglycemia would be greater in the diabetic. Hypoglycemia in diabetic patients sometimes is followed by an excessive response from the contra-insulin mechanism (probably including the pituitary, thyroid and adrenal), with an excessive glycosuria. Possibly in such cases repeated insulin shock therapy might lead to higher blood sugar levels and relative resistance to insulin.

Answers by Francis D. W. Lukens, Philadelphia, Pa.

QUESTION: Can one produce diabetes in the hypophysectomized animal with ACTH? With cortisone?

ANSWER: For both corticotropin and cortisone, the answer is "No." In connection with

such an answer, one should recall two facts: a) There is a great difference between species. Rats have been made temporarily diabetic with corticotropin or cortisone (Ingle *et al.*); dogs and cats cannot be made diabetic. In the majority of patients treated with these hormones diabetes does not develop; those patients in whom it does develop appear to have a significant past or family history of diabetes. b) Corticotropin or cortisone provides only partial replacement of pituitary insufficiency. In the absence of somatotropin and thyrotropin, and in the presence of the normal pancreatic reserve, one would not expect such a drastic overdosage effect as diabetes to occur. I can recall no references to formal efforts to produce diabetes in hypophysectomized animals with these agents.

QUESTION: Apparently globin insulin is not used a great deal. In daily dosage, *e.g.*, 30 units or less, do you see any particular advantage or disadvantage over NPH insulin.

ANSWER: Most authorities agree that globin insulin and NPH insulin are almost identical in their effects on the blood sugar level, but in practice certain differences are described. The advantages of globin insulin are that it causes less allergy than any of the insulins combined with protamine (PZI or NPH) and that it appears to be slightly more rapid in its action than NPH, and thus useful in some patients who might have reactions during the night. The disadvantage is said to be that, when used in combination with protamine or regular insulin, its action is less predictable or reliable. This would be difficult to measure amid the irregularities introduced by most out-patients. However, in the doses of 30 units or less suggested in the question, there should be no important difference between NPH and globin insulin. (Incidentally, at that dosage level in mild adult diabetics, one dose of regular insulin worked well before the depot insulins were developed.)

QUESTION: Do coronary occlusions with myocardial infarctions occur in middle-aged persons receiving insulin-shock therapy?

ANSWER: It seems agreed that insulin reaction or shock therapy does not exert any persistent change in the normal heart, although transient changes, such as inversion of the T-waves, may occur. No electrocardiographic or other changes have been found in young schizophrenic patients after long courses of shock treatment. It is difficult to secure information on middle-aged persons given shock therapy. One consultant has pointed out the fact that at his institution insulin-shock therapy is rarely used in patients older than 40 or 45 years, but that electroshock has been used up to the age of 80 years. Since both must cause some reflex secretion of epinephrine, there must be other reasons for this practice. Known coronary disease would be a contraindication to shock therapy in most patients. Apparently there is no unusual incidence of coronary occlusion when otherwise normal middle-aged patients are given shock therapy.

Answers by Ray F. Farquharson, Toronto, Canada

QUESTION: Is there any explanation for the localized obesity (buffalo hump) of Cushings syndrome?

ANSWER: I know of no explanation for the localized obesity (buffalo hump) of Cushing's syndrome.

QUESTION: Please discuss the use of cortisone or ACTH in gouty arthritis.

ANSWER: The use of cortisone and ACTH in treatment of gouty arthritis is not as beneficial as was earlier hoped. Cortisone is of little value; it is not very effective in the acute attack and exacerbations have occurred while the patient remained on active treatment.

Acute attacks may, however, be relieved dramatically by ACTH therapy, either when 25 to 50 mg. (units) is given intramuscularly four times a day or when 20 mg. is given each day over an eight-hour period in a continuous intravenous infusion. Unfortunately there is often an acute relapse on termination of the ACTH administration. For this reason it is commonly recommended that 1 mg. of colchicine be given by mouth three times a day during the period of ACTH therapy and for several days afterwards. This is usually effective.

It is doubtful, however, whether the use of ACTH is any better than adequate colchicine therapy. Colchicine may be given by mouth, either in a dosage of 1 mg. every two hours for five doses or 0.5 mg. hourly for ten doses; if the gouty arthritis is still active, the same dosage should be continued until the symptoms begin to subside or until toxic symptoms such as diarrhea, or nausea and vomiting appear. Alternatively, colchicine may be given intravenously during the acute attack; one injection of 3 mg. will commonly suffice but occasionally a second injection may be required; usually there are no toxic symptoms.

The administration of phenylbutazone (Butazolidin), in a dosage of 200 mg. given by mouth every six hours for three or four days, appears to be about as effective as colchicine in relieving an acute attack. Many patients prefer it, because it is well tolerated. It is virtually free from toxic effects if given for only a few days.

QUESTION: What are the actual results of stimulating the pituitary gland with roentgen therapy?

ANSWER: There is no clear, definite knowledge concerning the effect of radiation on the pituitary gland. Almost all experience has had to do with large-dosage radiation, given to reduce the overactivity of eosinophilic adenomas in acromegalics or to obtain similar effects on the pituitary gland in patients with Cushing's syndrome or hyperthyroidism. Even in these conditions the effects have been extremely variable and beneficial results often of short duration. Whether the pituitary gland can be effectively stimulated by radiation is not known.

Answers by Paul Starr, Los Angeles, California

QUESTION: How important are emotional factors in the development of hyperthyroidism?

ANSWER: It seems certain that emotional factors, such as grief and resentment, are important in the development of some cases of hyperthyroidism. Personal situations that are intolerable to the patient apparently result in thyrotoxicosis. A case history in any series of patients with this disease, especially if the history is taken with this in mind, will indicate that emotional factors are very common. It also seems that persistence of the hyperthyroidism and resistance to cure are aggravated by emotional factors.

QUESTION: Comment on the electrocardiographic flat T waves which occur following the

intravenous injection of a solution of potassium chloride in myxedematous patients not receiving thyroid medication.

ANSWER: The question implies that the flat T waves seen in myxedema are due to potassium chloride injection. Flat T waves are known to occur in myxedema, per se. Hyperpotassemia would be expected to elevate the flat T wave as seen in myexdema, since it is known that the inverted T waves of myocardial infarction became elevated following intravenous potassium chloride. Further, alterations in the digitalis T waves are seen with hyperpotassemia. I know of no work at this time which has shown specifically that potassium chloride influences the flat T waves seen in hypothyroidism.

QUESTION: Do the responses to oral thyroid medication change with the seasons?

ANSWER: No variation in response to oral thyroid medication has been ascertained with the change of seasons. It is well to remember that in many cases a gradual loss of response to a particular dose level of thyroid medication may occur with the passage of time, causing the requirement to double or quadruple.

QUESTION: In evaluating PBI results, who determines whether a patient is euthyroid, hyperthyroid, or hypothyroid?

ANSWER: The serum PBI value itself, with the determination carried out by an experienced worker in a reliable laboratory and interpreted with all causes of clinical and chemical variation of iodine in mind, is at present the best evaluation of whether a patient is euthyroid, hyperthyroid, or hypothyroid.

QUESTION: Do you recommend the use of a small dose of sedative in preparation for a basal metabolism determination?

ANSWER: A dose of three quarters of a grain of Seconal or one-half grain of phenobarbital taken one hour before a basal metabolism test is advisable. It must be remembered that the basal metabolic rate determination is not a specific diagnostic procedure.

QUESTION: What is the value of a BMR determination in a hospital patient who has been given a long-acting barbiturate at bedtime and a rapid-acting barbiturate if awake before the BMR next morning?

ANSWER: The administration of a rapid-acting barbiturate, if the patient awakes before the basal metabolism test, is a desirable procedure.

QUESTION: What is the value of a BMR under anesthesia?

ANSWER: Basal metabolic rates determined under complete anesthesia will require statistical standardization before the value can be determined.

QUESTION: Please discuss the reliability of the blood cholesterol determination as a chemical test.

ANSWER: Blood cholesterol concentration has a wide normal variation, but in proper hands, is a reliable chemical test. The degree of true clinical variation is more of a handicap to diagnosis than is the laboratory error.

The Endocrine Society

THE 1954 ANNUAL MEETING

The Thirty-sixth Annual Meeting of The Endocrine Society will be held in the Sir Francis Drake Hotel, San Francisco, California, Thursday, Friday and Saturday, June 17–18–19, 1954.

Dr. Roberto F. Escamilla, 655 Sutter Street, San Francisco, California, is in charge of the local arrangements for the meeting.

All Scientific Sessions will be held in the Sir Francis Drake Hotel. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The annual dinner is scheduled for Friday, June 19, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the Sir Francis Drake Hotel, advising time of arrival and departure. Make your reservations now and avoid disappointment.

Those wishing to present papers, which will be limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. George W. Thorn, Peter Bent Brigham Hospital, Boston, Massachusetts, *not later than February 1, 1954*. It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and Journals of the Society. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each nonmember-author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.

Line 3. Institution of origin and city in which institution is located.

3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
4. Abstracts should be letter-perfect, since there will be no opportunity for proof reading by authors.

1954 AWARDS AND FELLOWSHIPS

Awards and Fellowships presented by The Endocrine Society carry no obligation by the recipient to the Society or to the donors.

Fellowships

Society Fellowships are designed to assist men or women of exceptional promise in furthering their educational training and advancement toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D. or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $5,000 may be divided into two Fellowships in varying amounts, in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

The Ayerst, McKenna and Harrison Fellowship was first awarded in 1947 to Dr. Samuel Dvoskin and in 1948 to Dr. Ernest M. Brown, Jr. Dr. Brown was re-elected in 1949. The Fellowship in 1950 was awarded to Dr. Lawrence E. Shulman and in 1952 to Dr. John C. Laidlaw. In 1954 it will be in the amount of $5,000.

THE SCHERING FELLOWSHIP IN ENDOCRINOLOGY

This Fellowship will not be awarded in 1954 in order that it may accumulate toward a $5,000 Fellowship to be awarded in 1955.

Award

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishment of an investigator, not more than thirty-five years of age, in the field of clinical

or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; in 1949 to Dr. George Sayers; in 1950 to Dr. Oscar M. Hechter; in 1951 to Dr. Albert Segaloff. Effective in 1952, the Award was increased from $1,200 to $1,800 and the recipient was Dr. Seymour Lieberman; in 1953 the Award was presented to Drs. Sidney Roberts and Clara Szego. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

Nominations

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in, endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma, and returned to him not later than *March 1, 1954.*

The American Goiter Association

THE 1954 ANNUAL MEETING

The next Annual Meeting of the American Goiter Association will be held in the Hotel Somerset, Boston, Massachusetts, April 29, 30 and May 1, 1954.

VAN METER PRIZE AWARD FOR 1954

The American Goiter Association again offers the Van Meter Prize Award of Three Hundred Dollars and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The award will be made at the annual meeting of the Association which will be held in Boston, Massachusetts, April 29, 30 and May 1, 1954, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English, and a typewritten double-spaced copy in duplicate sent to the Corresponding Secretary, Dr. John C. McClintock, 149½ Washington Avenue, Albany 10, New York, not later than *January 15, 1954*. The committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for the presentation of the Prize Award Essay by the author, if it is possible for him to attend. The essay will be published in the annual Transactions of the Association.

AWARD OF MERIT FOR 1954

TO ALL MEMBERS OF THE AMERICAN GOITER ASSOCIATION

An anonymous gift of $1,000 to be awarded each year for three years has been accepted by the Executive Council of The American Goiter Association. The terms of the gift specify that this sum shall be given to a member of the Association under 45 years of age who has made the most important contributions to the knowledge and understanding of thyroid disease or the treatment of thyroid disease.

Candidates for this award are to be nominated by members of the Association. When making a nomination, complete details of the nominee's present and past positions and training must be submitted. A complete bibliography of the nominee's publications is required. A full explanation of the reasons for nominating the candidate must be given.

The candidates submitted are to be reviewed by an anonymous committee appointed by the president. The chairman of this committee will present the deliberations and decisions of said committee to the Executive Council in advance of the annual meeting.

The award is to be called The American Goiter Association Award of Merit and shall be presented at the annual banquet.

You are requested to send two copies of your nominations to this secretary, making certain the information required by the deed of gift is included. All nominations must be received not later than *February 1, 1954.*

JOHN C. MCCLINTOCK, M.D.,
Recording and Corresponding Secretary

149½ Washington Avenue,
Albany 10, N. Y.

BOOKS RECEIVED

Atlas of Tumor Pathology. Published by the ARMED FORCES INSTITUTE OF PATHOLOGY, under the auspices of the Subcommittee on Oncology of the Committee on Pathology of the National Research Council, Washington, D. C.

Tumors of the Adrenal. (Section VIII, Fascicle 29.) By HOWARD T. KARSNER, M.D., Medical Research Advisor, Bureau of Medicine and Surgery, U. S. Navy; formerly Professor of Pathology, Western Reserve University, Cleveland, Ohio; 60 pages; 47 figures; 5 color plates; 1950. Price $1.00.

Tumors of the Parathyroid Glands. (Section IV, Fascicle 15.) By BENJAMIN CASTLEMAN, M.D., Acting Chief, Department of Pathology and Bacteriology, Massachusetts General Hospital, and Assistant Professor of Pathology, Harvard Medical School, Boston, Mass; 74 pages; 53 figures; 3 color plates; 1952. Price $0.65.

Tumors of the Thyroid Gland. (Section IV, Fascicle 14.) By SHIELDS WARREN, M.D., Professor of Pathology, Harvard Medical School, Pathologist, New England Deaconess Hospital, Boston, Mass., and WILLIAM A. MEISSNER, M.D., Assistant Professor of Pathology, Harvard Medical School, Associate Pathologist, New England Deaconess Hospital; 97 pages; 82 figures; 1 color plate; 1953. Price $1.75.

L'exploration fonctionelle de la cortico-surrénale par les stéroïdes. Par JEAN SCHWARTZ, Chef de clinique médicale à la Faculté de médecine de Strasbourg. Préface du Pr. J. Warter; avant-propos du Pr. G. Pincus; 164 pages; 17 figures; 1953. Masson et Cie, Paris. 960 fr.

Long-term Diabetes. The Clinical Picture in Diabetes Mellitus of 15–25 Years' Duration, with a Follow-up of a Regional Series of Cases. By KNUD LUNDBAEK, M.D. Copenhagen, Lecturer in Internal Medicine, Aarhus University Medical School Ophthalmological Section, in collaboration with VIGGO A. JENSEN, M.D., Professor of Ophthalmology, Aarhus University Medical School; translated from the Danish by AXEL ANDERSEN, Copenhagen; 197 pages; 33 figures; 25 tables; 1953. Ejnar Munksgaard, Copenhagen, Denmark. D.Kr. 32/-.

Major Metabolic Fuels. No. 5 of a series of symposia sponsored by the Biology Department, Brookhaven National Laboratory. This volume records a symposium held September 3–5, 1952; 234 pages; published 1953. Upton, New York, Brookhaven National Laboratory. Available from the Office of Technical Services, Department of Commerce, Washington 25, D. C. Price $1.35.

Memoirs of the Society for Endocrinology No. 1. The Thyroid Gland. A record of a Symposium held jointly by the Society for Endocrinology and the Endocrinological Section of the Royal Society of Medicine, London, Feb. 25, 1953. Available to American purchasers from Pitman Publishing Corporation, 2 West 45th Street, New York 26. Price $1.00 plus postage.

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